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(54) Title: CYTOKINE-RECEPTOR EXPRESSED IN TESTIS CELLS

(57) Abstract

Novel receptor polypeptides, polynucleotides encoding the polypeptides, and related compositions and methods are disclosed. The polypeptides comprise an extracellular domain of a cell-surface receptor that is expressed in testis cells. The polypeptides may be used within methods for detecting ligands that promote the proliferation and/or differentiation of testis cells, and may also be used in the development of male-specific contraceptives and infertility treatments.

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Description

CYTOKINE-RECEPTOR EXPRESSED IN TESTIS CELLS

Background of the Invention

Proliferation and differentiation of cells of multicellular organisms are controlled by hormones and polypeptide growth factors. These diffusable molecules allow cells to communicate with each other and act in concert to form cells and organs, and to repair and regenerate damaged tissue. Examples of hormones and growth factors include the steroid hormones (e.g. estrogen, testosterone), parathyroid hormone, follicle stimulating hormone, the interleukins, platelet derived growth factor (PDGF), epidermal growth factor (EGF), granulocyte-macrophage colony stimulating factor (GM-CSF), erythropoietin (EPO) and calcitonin.

Hormones and growth factors influence cellular metabolism by binding to receptors. Receptors may be integral membrane proteins that are linked to signalling pathways within the cell, such as second messenger systems. Other classes of receptors are soluble molecules, such as the transcription factors.

Of particular interest are receptors for cytokines, molecules that promote the proliferation and/or differentiation of cells. Examples of cytokines include erythropoietin (EPO), which stimulates the development of red blood cells; thrombopoietin (TPO), which stimulates development of cells of the megakaryocyte lineage; and granulocyte-colony stimulating factor (G-CSF), which stimulates development of neutrophils. These cytokines are useful in restoring normal blood cell levels in patients suffering from anemia or receiving chemotherapy for cancer. The demonstrated *in vivo* activities of these cytokines illustrates the enormous clinical potential of, and need for, other cytokines, cytokine agonists, and

cytokine antagonists. The present invention addresses this need by providing novel cytokine receptors and related compositions and methods.

Summary of the Invention

Within one aspect, the present invention provides an isolated polynucleotide encoding a ligand-binding receptor polypeptide. The polypeptide comprises a sequence of amino acids selected from the group consisting of (a) residues 141 to 337 of SEQ ID NO:2; (b) allelic variants of (a); and (c) sequences that are at least 80% identical to (a) or (b). Within one embodiment, the polypeptide comprises residues 141 to 337 of SEQ ID NO:2 or SEQ ID NO:4. Within another embodiment, the polypeptide encoded by the isolated polynucleotide further comprises a transmembrane domain. The transmembrane domain may comprise residues 340 to 363 of SEQ ID NO:2, or an allelic variant thereof. Within another embodiment, the polypeptide encoded by the isolated polynucleotide further comprises an intracellular domain, such as an intracellular domain comprising residues 364 to 380 of SEQ ID NO:2, or an allelic variant thereof. Within further embodiments, the polynucleotide encodes a polypeptide that comprises residues 25 to 337, 1 to 337, or 1 to 380 of SEQ ID NO:2 or SEQ ID NO:4. Within an additional embodiment, the polypeptide further comprises an affinity tag. Within a further embodiment, the polynucleotide is DNA.

Within a second aspect of the invention there is provided an expression vector comprising (a) a transcription promoter; (b) a DNA segment encoding a secretory peptide and a ligand-binding receptor polypeptide, wherein the polypeptide comprises a sequence of amino acids selected from the group consisting of: (i) residues 141 to 337 of SEQ ID NO:2; (ii) allelic variants of (i); and (iii) sequences that are at least 80% identical to (i) or (ii); and (c) a transcription terminator, wherein the promoter, DNA segment, and

terminator are operably linked. The ligand-binding receptor polypeptide may further comprise a transmembrane domain, or a transmembrane domain and an intracellular domain.

Within a third aspect of the invention there is provided a cultured eukaryotic cell into which has been introduced an expression vector as disclosed above, wherein said cell expresses a receptor polypeptide encoded by the DNA segment. Within one embodiment, the cell further expresses a signalling subunit, such as a hematopoietic receptor β_C subunit. Within another embodiment, the cell is dependent upon an exogenously supplied hematopoietic growth factor for proliferation.

Within a fourth aspect of the invention there is provided an isolated polypeptide comprising a segment selected from the group consisting of (a) residues 141 to 337 of SEQ ID NO:2; (b) allelic variants of (a); and (c) sequences that are at least 80% identical to (a) or (b), wherein said polypeptide is substantially free of transmembrane and intracellular domains ordinarily associated with hematopoietic receptors. Within one embodiment, the polypeptide further comprises an immunoglobulin F_C polypeptide. Within a another embodiment, the polypeptide further comprises an affinity tag, such as polyhistidine, protein A, glutathione S transferase, or an immunoglobulin heavy chain constant region. Within a further embodiment, the polypeptide comprises residues 25-337 of SEQ ID NO:2, an allelic variant of SEQ ID NO:2, or a sequence that is at least 80% identical to residues 25-337 of SEQ ID NO:2 or an allelic variant of SEQ ID NO:2.

Within a further aspect of the invention there is provided a chimeric polypeptide consisting essentially of a first portion and a second portion joined by a peptide bond. The first portion of the chimeric polypeptide consists essentially of a ligand binding domain of a receptor polypeptide selected from the group

consisting of (a) a receptor polypeptide as shown in SEQ ID NO:2; (b) allelic variants of SEQ ID NO:2; and (c) receptor polypeptides that are at least 80% identical to (a) or (b). The second portion of the chimeric polypeptide consists essentially of an affinity tag. Within one embodiment the affinity tag is an immunoglobulin F_C polypeptide. The invention also provides expression vectors encoding the chimeric polypeptides and host cells transfected to produce the chimeric polypeptides.

The invention also provides a method for detecting a ligand within a test sample, comprising contacting a test sample with a polypeptide as disclosed above, and detecting binding of the polypeptide to ligand in the sample. Within one embodiment the polypeptide further comprises transmembrane and intracellular domains. The polypeptide can be membrane bound within a cultured cell, wherein the detecting step comprises measuring a biological response in the cultured cell. Within another embodiment, the polypeptide is immobilized on a solid support.

Within an additional aspect of the invention there is provided an antibody that specifically binds to a polypeptide as disclosed above.

These and other aspects of the invention will become evident upon reference to the following detailed description and the attached drawing.

Brief Description of the Drawing

The Figure illustrates conserved structural features in cytokine receptors.

Detailed Description of the Invention

The term "allelic variant" is used herein to denote any of two or more alternative forms of a gene occupying the same chromosomal locus. Allelic variation arises naturally through mutation, and may result in

phenotypic polymorphism within populations. Gene mutations can be silent (no change in the encoded polypeptide) or may encode polypeptides having altered amino acid sequence. The term allelic variant is also used herein to denote a protein encoded by an allelic variant of a gene.

The term "expression vector" is used to denote a DNA molecule, linear or circular, that comprises a segment encoding a polypeptide of interest operably linked to additional segments that provide for its transcription. Such additional segments include promoter and terminator sequences, and may also include one or more origins of replication, one or more selectable markers, an enhancer, a polyadenylation signal, etc. Expression vectors are generally derived from plasmid or viral DNA, or may contain elements of both.

The term "isolated", when applied to a polynucleotide, denotes that the polynucleotide has been removed from its natural genetic milieu and is thus free of other extraneous or unwanted coding sequences, and is in a form suitable for use within genetically engineered protein production systems.

"Operably linked", when referring to DNA segments, indicates that the segments are arranged so that they function in concert for their intended purposes, e.g. transcription initiates in the promoter and proceeds through the coding segment to the terminator.

A "polynucleotide" is a single- or double-stranded polymer of deoxyribonucleotide or ribonucleotide bases read from the 5' to the 3' end. Polynucleotides include RNA and DNA, and may be isolated from natural sources, synthesized *in vitro*, or prepared from a combination of natural and synthetic molecules.

The term "promoter" is used herein for its art-recognized meaning to denote a portion of a gene containing DNA sequences that provide for the binding of RNA polymerase and initiation of transcription. Promoter

sequences are commonly, but not always, found in the 5' non-coding regions of genes.

The term "receptor" is used herein to denote a cell-associated protein, or a polypeptide subunit of such a protein, that binds to a bioactive molecule (the "ligand") and mediates the effect of the ligand on the cell. Binding of ligand to receptor results in a conformational change in the receptor (and, in some cases, receptor multimerization, i.e., association of identical or different receptor subunits) that causes interactions between the effector domain(s) and other molecule(s) in the cell. These interactions in turn lead to alterations in the metabolism of the cell. Metabolic events that are linked to receptor-ligand interactions include gene transcription, phosphorylation, dephosphorylation, cell proliferation, increases in cyclic AMP production, mobilization of cellular calcium, mobilization of membrane lipids, cell adhesion, hydrolysis of inositol lipids and hydrolysis of phospholipids. The term "receptor polypeptide" is used to denote complete receptor polypeptide chains and portions thereof, including isolated functional domains (e.g., ligand-binding domains).

A "secretory signal sequence" is a DNA sequence that encodes a polypeptide (a "secretory peptide") that, as a component of a larger polypeptide, directs the larger polypeptide through a secretory pathway of a cell in which it is synthesized. The larger polypeptide is commonly cleaved to remove the secretory peptide during transit through the secretory pathway.

A "soluble receptor" is a receptor polypeptide that is not bound to a cell membrane. Soluble receptors are most commonly ligand-binding receptor polypeptides that lack transmembrane and cytoplasmic domains. Soluble receptors can comprise additional amino acid residues, such as affinity tags that provide for purification of the polypeptide or provide sites for attachment of the

polypeptide to a substrate, or immunoglobulin constant region sequences. Many cell-surface receptors have naturally occurring, soluble counterparts that are produced by proteolysis or translated from alternatively spliced mRNAs. Receptor polypeptides are said to be substantially free of transmembrane and intracellular polypeptide segments when they lack sufficient portions of these segments to provide membrane anchoring or signal transduction, respectively.

The present invention is based in part upon the discovery of a novel DNA sequence that encodes a protein having the structure of a cytokine receptor, including the conserved WSXWS motif (SEQ ID NO:5). Analysis of the tissue distribution of the mRNA corresponding to this novel DNA showed that it was highly expressed in the testes, suggesting that the receptor mediates processes of progenitor cell growth and development, such as spermatogenesis. The receptor is also expressed at lower levels in pituitary. Subsequently, the receptor was shown to bind interleukin 13 (IL-13). The human cDNA was subsequently used to clone the orthologous receptor from Celebus macaque. The receptor has been designated "ZCytor2".

Cytokine receptors subunits are characterized by a multi-domain structure comprising a ligand-binding domain and an effector domain that is typically involved in signal transduction. Multimeric cytokine receptors include homodimers (e.g., PDGF receptor αα and ββ isoforms, erythropoietin receptor, MPL [thrombopoietin receptor], and G-CSF receptor), heterodimers whose subunits each have ligand-binding and effector domains (e.g., PDGF receptor αβ isoform), and multimers having component subunits with disparate functions (e.g., IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, and GM-CSF receptors). Some receptor subunits are common to a plurality of receptors. For example, the AIC2B subunit, which cannot bind ligand on its own but includes an intracellular signal

transduction domain, is a component of IL-3 and GM-CSF receptors. Many cytokine receptors can be placed into one of four related families on the basis of their structures (see Figure) and functions. Hematopoietic receptors, for example, are characterized by the presence of a domain containing conserved cysteine residues and the WSXWS motif (SEQ ID NO:5). Additional domains, including protein kinase domains; fibronectin type III domains; and immunoglobulin domains, which are characterized by disulfide-bonded loops, are present in certain hematopoietic receptors. Cytokine receptor structure has been reviewed by Urdal, Ann. Reports Med. Chem. 26:221-228, 1991 and Cosman, Cytokine 5:95-106, 1993. It is generally believed that under selective pressure for organisms to acquire new biological functions, new receptor family members arose from duplication of existing receptor genes leading to the existence of multi-gene families. Family members thus contain vestiges of the ancestral gene, and these characteristic features can be exploited in the isolation and identification of additional family members. The cytokine receptor superfamily is subdivided as shown in Table 1.

Table 1

Cytokine Receptor Superfamily

Immunoglobulin family
- CSF-1 receptor
- MGF receptor
- IL-1 receptor
- PDGF receptor

Hematopoietin family
- erythropoietin receptor
- G-CSF receptor
- IL-2 receptor β-subunit
- IL-3 receptor

Table 1. continued

	IL-4 receptor
	IL-5 receptor
	IL-6 receptor
	IL-7 receptor
	IL-9 receptor
	GM-CSF receptor α-subunit
	GM-CSF receptor β-subunit
	Prolactin receptor
	CNTF receptor
	Oncostatin M receptor
	Leukemia inhibitory factor receptor
	Growth hormone receptor
	MPL
	Leptin receptor
TNF receptor family	
	TNF (p80) receptor
	TNF (p60) receptor
	TNFR-RP
	CD27
	CD30
	CD40
	4-1BB
	OX-40
	Fas
	NGF receptor
Other	
	IL-2 receptor α-subunit
	IL-15 receptor α-subunit
	IFN-γ receptor

Cell-surface cytokine receptors are further characterized by the presence of additional domains. These receptors are anchored in the cell membrane by a transmembrane domain characterized by a sequence of hydrophobic amino acid residues (typically about 21-25 residues), which is commonly flanked by positively charged

residues (Lys or Arg). On the opposite end of the protein from the extracellular domain and separated from it by the transmembrane domain is an intracellular domain.

The novel receptor of the present invention was initially identified by the presence of the conserved WSXWS motif (SEQ ID NO:5). Analysis of a human cDNA clone encoding ZCytor2 (SEQ ID NO:1) revealed an open reading frame encoding 380 amino acids (SEQ ID NO:2) comprising an extracellular ligand-binding domain of approximately 315 amino acid residues (residues 25-339 of SEQ ID NO:2), a transmembrane domain of approximately 24 amino acid residues (residues 340-363 of SEQ ID NO:2), and a short intracellular domain of approximately 17 amino acid residues (residues 364-380 of SEQ ID NO:2). Those skilled in the art will recognize that these domain boundaries are approximate and are based on alignments with known proteins and predictions of protein folding. Deletion of residues from the ends of the domains is possible. For example, the core ligand binding region is believed to reside within residues 141-337 of SEQ ID NO:2. Structural analysis indicates that the polypeptide regions from Cys145 through Cys155 and from Cys184 through Cys197 of SEQ ID NO:2 are cysteine loops that are important ligand-binding sites. Relatively small, ligand-binding receptor polypeptides are thus provided by the present invention.

The deduced amino acid sequence of Zcytor2 indicates that it belongs to the same subfamily as the IL-3, IL-5 and GM-CSF receptor α subunits. These α receptor subunits are ligand-specific proteins that combine with a common signalling subunit (β-subunit) to form a signalling complex in the presence of the cognate ligand. The β-subunit for this receptor subfamily has been previously identified in mouse (Itoh et al., Science 247:324-327, 1989; Gorman et al., Proc. Natl. Acad. Sci. USA 87:5459-5463, 1990) and human (Hayashida, et al., Proc. Natl. Aca. Sci. USA 87:9655-9659, 1990). The mouse β-subunit occurs in two isoforms, denoted AIC2A and AIC2B, whereas in human

only one form (denoted β_c) has been identified. β_c is also a member of the hematopoietin receptor family in that it contains a WSXWS motif (SEQ ID NO:5) and a single transmembrane domain. β_c also contains a sizable intracellular domain capable of interacting with cytoplasmic proteins for signal propagation. In the alternative, Zcytor2 may combine with one or more of gp130 (Hibi et al., Cell 63:1149-1157, 1990), the IL-4 α-subunit (Idzerda, et al., J. Exp. Med. 171:861, 1990), or the IL-13 α-subunit (Hilton et al., Proc. Natl. Acad. Sci. USA 93:497-501, 1996) in a tissue specific manner to form dimeric or trimeric complexes. Binding data for Zcytor2 suggest that this receptor subunit may form an IL-13 receptor complex in testes and pituitary that is different from the immune system IL-13 receptor.

Within preferred embodiments of the invention the isolated polynucleotides will hybridize to similar sized regions of SEQ ID NO:1, SEQ ID NO:3, or SEQ ID NO:6, or a sequence complementary thereto, under stringent conditions. In general, stringent conditions are selected to be about 5°C lower than the thermal melting point (T_m) for the specific sequence at a defined ionic strength and pH. The T_m is the temperature (under defined ionic strength and pH) at which 50% of the target sequence hybridizes to a perfectly matched probe. Typical stringent conditions are those in which the salt concentration is at least about 0.02 M at pH 7 and the temperature is at least about 60°C. As previously noted, the isolated polynucleotides of the present invention include DNA and RNA. Methods for isolating DNA and RNA are well known in the art. It is generally preferred to isolate RNA from testis, including whole testis tissue extracts or testicular cells, such as Sertoli cells, Leydig cells, spermatogonia, or epididymis, although DNA can also be prepared using RNA from other tissues or isolated as genomic DNA. Total RNA can be prepared using guanidine HCl extraction followed by isolation by

centrifugation in a CsCl gradient (Chirgwin et al., Biochemistry 18:52-94, 1979). Poly (A)+ RNA is prepared from total RNA using the method of Aviv and Leder (Proc. Natl. Acad. Sci. USA 69:1408-1412, 1972). Complementary DNA (cDNA) is prepared from poly(A)+ RNA using known methods. Polynucleotides encoding Zcytor2 polypeptides are then identified and isolated by, for example, hybridization or PCR.

Those skilled in the art will recognize that the sequences disclosed in SEQ ID NOS:1, 2, 6, and 7 represent single alleles of the human and macaque ZCytor2 receptors, respectively. Allelic variants of these sequences can be cloned by probing cDNA or genomic libraries from different individuals according to standard procedures. DNA and protein sequences from an additional human clone are shown in SEQ ID NOS: 3 and 4.

The present invention further provides counterpart receptors and polynucleotides from other species ("species orthologs"). Of particular interest are ZCytor2 receptors from other mammalian species, including murine, porcine, ovine, bovine, canine, feline, equine, and other primate receptors. Species orthologs of the human and macaque ZCytor2 receptors can be cloned using information and compositions provided by the present invention in combination with conventional cloning techniques. For example, a cDNA can be cloned using mRNA obtained from a tissue or cell type that expresses the receptor. Suitable sources of mRNA can be identified by probing Northern blots with probes designed from the sequences disclosed herein. A library is then prepared from mRNA of a positive tissue or cell line. A receptor-encoding cDNA can then be isolated by a variety of methods, such as by probing with a complete or partial human or macaque cDNA or with one or more sets of degenerate probes based on the disclosed sequences. A cDNA can also be cloned using the polymerase chain reaction, or PCR (Mullis, U.S. Patent No. 4,683,202).

using primers designed from the sequences disclosed herein. Within an additional method, the cDNA library can be used to transform or transfect host cells, and expression of the cDNA of interest can be detected with an antibody to the receptor. Similar techniques can also be applied to the isolation of genomic clones.

The present invention also provides isolated receptor polypeptides that are substantially homologous to the receptor polypeptides of SEQ ID NO: 2 or SEQ ID NO:7 and their species orthologs. By "isolated" is meant a protein or polypeptide that is found in a condition other than its native environment, such as apart from blood and animal tissue. In a preferred form, the isolated polypeptide is substantially free of other polypeptides, particularly other polypeptides of animal origin. It is prefered to provide the polypeptides in a highly purified form, i.e. greater than 95% pure, more preferably greater than 99% pure. The term "substantially homologous" is used herein to denote polypeptides having 50%, preferably 60%, more preferably at least 80%, sequence identity to the sequences shown in SEQ ID NO:2, 4, or 7 or their species orthologs. Such polypeptides will more preferably be at least 90% identical, and most preferably 95% or more identical to SEQ ID NO:2, 4 or 7 or their species orthologs. Percent sequence identity is determined by conventional methods. See, for example, Altschul et al., Bull. Math. Bio. 48: 603-616, 1986 and Henikoff and Henikoff, Proc. Natl. Acad. Sci. USA 89:10915-10919, 1992. Briefly, two amino acid sequences are aligned to optimize the alignment scores using a gap opening penalty of 10, a gap extension penalty of 1, and the "blosum 62" scoring matrix of Henikoff and Henikoff (ibid.) as shown in Table 2 (amino acids are indicated by the standard one-letter codes). The percent identity is then calculated as:

$$\frac{\text{Total number of identical matches}}{[\text{length of the longer sequence plus the}} \times 100$$

number of gaps introduced into the longer sequence in order to align the two sequences]

Table 2

	A	R	N	D	C	Q	E	G	H	I	L	K	M	F	P	S	T	W	Y	V
A	4																			
R	-1	5																		
N	-2	0	6																	
D	-2	-2	1	6																
C	0	-3	-3	-3	9															
Q	-1	1	0	0	-3	5														
E	-1	0	0	2	-4	2	5													
G	0	-2	0	-1	-3	-2	-2	6												
H	-2	0	1	-1	-3	0	0	-2	8											
I	-1	-3	-3	-3	-1	-3	-3	-4	-3	4										
L	-1	-2	-3	-4	-1	-2	-3	-4	-3	2	4									
K	-1	2	0	-1	-3	1	1	-2	-1	-3	-2	5								
M	-1	-1	-2	-3	-1	0	-2	-3	-2	1	2	-1	5							
F	-2	-3	-3	-3	-2	-3	-3	-3	-1	0	0	-3	0	6						
P	-1	-2	-2	-1	-3	-1	-1	-2	-2	-3	-3	-1	-2	-4	7					
S	1	-1	1	0	-1	0	0	0	-1	-2	-2	0	-1	-2	-1	4				
T	0	-1	0	-1	-1	-1	-1	-2	-2	-1	-1	-1	-1	-2	-1	1	5			
W	-3	-3	-4	-4	-2	-2	-3	-2	-2	-3	-2	-3	-1	1	-4	-3	-2	11		
Y	-2	-2	-2	-3	-2	-1	-2	-3	2	-1	-1	-2	-1	3	-3	-2	-2	2	7	
V	0	-3	-3	-3	-1	-2	-2	-3	-3	3	1	-2	1	-1	-2	-2	0	-3	-1	4

Sequence identity of polynucleotide molecules is determined by similar methods using a ratio as disclosed above.

Substantially homologous proteins and polypeptides are characterized as having one or more amino acid substitutions, deletions or additions. These changes are preferably of a minor nature, that is conservative amino acid substitutions (see Table 3) and other substitutions that do not significantly affect the folding or activity of the protein or polypeptide; small deletions, typically of one to about 30 amino acids; and small amino- or carboxyl-terminal extensions, such as an amino-terminal methionine residue, a small linker peptide of up to about 20-25 residues, or a small extension that facilitates purification (an affinity tag), such as a poly-histidine tract, protein A (Nilsson et al., EMBO J. 4:1075, 1985; Nilsson et al., Methods Enzymol. 198:3, 1991), glutathione S transferase (Smith and Johnson, Gene 67:31, 1988), or other antigenic epitope or binding domain. See, in general Ford et al., Protein Expression and Purification 2: 95-107, 1991, which is incorporated herein by reference. DNAs encoding affinity tags are available from commercial suppliers (e.g., Pharmacia Biotech, Piscataway, NJ).

Table 3

Conservative amino acid substitutions

Basic:	arginine
	lysine
	histidine
Acidic:	glutamic acid
	aspartic acid
Polar:	glutamine
	asparagine
Hydrophobic:	leucine
	isoleucine
	valine

Table 3. continued

Aromatic:	phenylalanine
	tryptophan
	tyrosine
Small:	glycine
	alanine
	serine
	threonine
	methionine

Essential amino acids in the receptor polypeptides of the present invention can be identified according to procedures known in the art, such as site-directed mutagenesis or alanine-scanning mutagenesis (Cunningham and Wells, Science 244, 1081-1085, 1989; Bass et al., Proc. Natl. Acad. Sci. USA 88:4498-4502, 1991). In the latter technique, single alanine mutations are introduced at every residue in the molecule, and the resultant mutant molecules are tested for biological activity (e.g., ligand binding and signal transduction) to identify amino acid residues that are critical to the activity of the molecule. Sites of ligand-receptor interaction can also be determined by analysis of crystal structure as determined by such techniques as nuclear magnetic resonance, crystallography or photoaffinity labeling. See, for example, de Vos et al., Science 255:306-312, 1992; Smith et al., J. Mol. Biol. 224:899-904, 1992; Wlodaver et al., FEBS Lett. 309:59-64, 1992. The identities of essential amino acids can also be inferred from analysis of homologies with related receptors.

Multiple amino acid substitutions can be made and tested using known methods of mutagenesis and screening, such as those disclosed by Reidhaar-Olson and Sauer (Science 241:53-57, 1988) or Bowie and Sauer (Proc. Natl. Acad. Sci. USA 86:2152-2156, 1989). Briefly, these authors disclose methods for simultaneously randomizing

two or more positions in a polypeptide, selecting for functional polypeptide, and then sequencing the mutagenized polypeptides to determine the spectrum of allowable substitutions at each position. Other methods that can be used include phage display (e.g., Lowman et al., Biochem. 30:10832-10837, 1991; Ladner et al., U.S. Patent No. 5,223,409; Huse, WIPO Publication WO 92/06204) and region-directed mutagenesis (Derbyshire et al., Gene 46:145, 1986; Ner et al., DNA 7:127, 1988)

Mutagenesis methods as disclosed above can be combined with high-throughput screening methods to detect activity of cloned, mutagenized receptors in host cells. Preferred assays in this regard include cell proliferation assays and biosensor-based ligand-binding assays, which are described below. Mutagenized DNA molecules that encode active receptors or portions thereof (e.g., ligand-binding fragments) can be recovered from the host cells and rapidly sequenced using modern equipment. These methods allow the rapid determination of the importance of individual amino acid residues in a polypeptide of interest, and can be applied to polypeptides of unknown structure.

Using the methods discussed above, one of ordinary skill in the art can prepare a variety of polypeptides that are substantially homologous to residues 141 to 337 of SEQ ID NO:2 or allelic variants thereof and retain the ligand-binding properties of the wild-type receptor. Such polypeptides may include additional amino acids from an extracellular ligand-binding domain of a Zcytor2 receptor as well as part or all of the transmembrane and intracellular domains. Such polypeptides may also include additional polypeptide segments as generally disclosed above.

The receptor polypeptides of the present invention, including full-length receptors, receptor fragments (e.g. ligand-binding fragments), and fusion polypeptides can be produced in genetically engineered

host cells according to conventional techniques. Suitable host cells are those cell types that can be transformed or transfected with exogenous DNA and grown in culture, and include bacteria, fungal cells, and cultured higher eukaryotic cells. Eukaryotic cells, particularly cultured cells of multicellular organisms, are preferred. Techniques for manipulating cloned DNA molecules and introducing exogenous DNA into a variety of host cells are disclosed by Sambrook et al., Molecular Cloning: A Laboratory Manual, 2nd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989, and Ausubel et al., ibid., which are incorporated herein by reference.

In general, a DNA sequence encoding a ZCytor2 receptor polypeptide is operably linked to other genetic elements required for its expression, generally including a transcription promoter and terminator, within an expression vector. The vector will also commonly contain one or more selectable markers and one or more origins of replication, although those skilled in the art will recognize that within certain systems selectable markers may be provided on separate vectors, and replication of the exogenous DNA may be provided by integration into the host cell genome. Selection of promoters, terminators, selectable markers, vectors and other elements is a matter of routine design within the level of ordinary skill in the art. Many such elements are described in the literature and are available through commercial suppliers.

To direct a ZCytor2 receptor polypeptide into the secretory pathway of a host cell, a secretory signal sequence (also known as a leader sequence, prepro sequence or pre sequence) is provided in the expression vector. The secretory signal sequence may be that of the receptor, or may be derived from another secreted protein (e.g., t-PA) or synthesized *de novo*. The secretory signal sequence is joined to the ZCytor2 DNA sequence in the correct reading frame. Secretory signal sequences are commonly positioned 5' to the DNA sequence encoding the polypeptide

of interest, although certain signal sequences may be positioned elsewhere in the DNA sequence of interest (see, e.g., Welch et al., U.S. Patent No. 5,037,743; Holland et al., U.S. Patent No. 5,143,830).

Cultured mammalian cells are preferred hosts within the present invention. Methods for introducing exogenous DNA into mammalian host cells include calcium phosphate-mediated transfection (Wigler et al., Cell 14:725, 1978; Corsaro and Pearson, Somatic Cell Genetics 7:603, 1981: Graham and Van der Eb, Virology 52:456, 1973), electroporation (Neumann et al., EMBO J. 1:841-845, 1982), DEAE-dextran mediated transfection (Ausubel et al., eds., Current Protocols in Molecular Biology, John Wiley and Sons, Inc., NY, 1987), and liposome-mediated transfection (Hawley-Nelson et al., Focus 15:73, 1993; Ciccarone et al., Focus 15:80, 1993), which are incorporated herein by reference. The production of recombinant polypeptides in cultured mammalian cells is disclosed, for example, by Levinson et al., U.S. Patent No. 4,713,339; Hagen et al., U.S. Patent No. 4,784,950; Palmiter et al., U.S. Patent No. 4,579,821; and Ringold, U.S. Patent No. 4,656,134, which are incorporated herein by reference. Suitable cultured mammalian cells include the COS-1 (ATCC No. CRL 1650), COS-7 (ATCC No. CRL 1651), BHK (ATCC No. CRL 1632), BHK 570 (ATCC No. CRL 10314), 293 (ATCC No. CRL 1573; Graham et al., J. Gen. Virol. 36:59-72, 1977) and Chinese hamster ovary (e.g. CHO-K1; ATCC No. CCL 61) cell lines. Additional suitable cell lines are known in the art and available from public depositories such as the American Type Culture Collection, Rockville, Maryland. In general, strong transcription promoters are preferred, such as promoters from SV-40 or cytomegalovirus. See, e.g., U.S. Patent No. 4,956,288. Other suitable promoters include those from metallothionein genes (U.S. Patent Nos. 4,579,821 and 4,601,978, which are incorporated herein by reference) and the adenovirus major late promoter.

Drug selection is generally used to select for cultured mammalian cells into which foreign DNA has been inserted. Such cells are commonly referred to as "transfectants". Cells that have been cultured in the presence of the selective agent and are able to pass the gene of interest to their progeny are referred to as "stable transfectants." A preferred selectable marker is a gene encoding resistance to the antibiotic neomycin. Selection is carried out in the presence of a neomycin-type drug, such as G-418 or the like. Selection systems may also be used to increase the expression level of the gene of interest, a process referred to as "amplification." Amplification is carried out by culturing transfectants in the presence of a low level of the selective agent and then increasing the amount of selective agent to select for cells that produce high levels of the products of the introduced genes. A preferred amplifiable selectable marker is dihydrofolate reductase, which confers resistance to methotrexate. Other drug resistance genes (e.g. hygromycin resistance, multi-drug resistance, puromycin acetyltransferase) can also be used.

Other higher eukaryotic cells can also be used as hosts, including insect cells, plant cells and avian cells. Transformation of insect cells and production of foreign polypeptides therein is disclosed by Guarino et al., U.S. Patent No. 5,162,222; Bang et al., U.S. Patent No. 4,775,624; and WIPO publication WO 94/06463, which are incorporated herein by reference. The use of *Agrobacterium rhizogenes* as a vector for expressing genes in plant cells has been reviewed by Sinkar et al., J. Biosci. (Bangalore) 11:47-58, 1987.

Fungal cells, including yeast cells, and particularly cells of the genus *Saccharomyces*, can also be used within the present invention, such as for producing receptor fragments or polypeptide fusions. Methods for transforming yeast cells with exogenous DNA and producing

recombinant polypeptides therefrom are disclosed by, for example, Kawasaki, U.S. Patent No. 4,599,311; Kawasaki et al., U.S. Patent No. 4,931,373; Brake, U.S. Patent No. 4,870,008; Welch et al., U.S. Patent No. 5,037,743; and Murray et al., U.S. Patent No. 4,845,075, which are incorporated herein by reference. Transformed cells are selected by phenotype determined by the selectable marker, commonly drug resistance or the ability to grow in the absence of a particular nutrient (e.g., leucine). A preferred vector system for use in yeast is the POT1 vector system disclosed by Kawasaki et al. (U.S. Patent No. 4,931,373), which allows transformed cells to be selected by growth in glucose-containing media. Suitable promoters and terminators for use in yeast include those from glycolytic enzyme genes (see, e.g., Kawasaki, U.S. Patent No. 4,599,311; Kingsman et al., U.S. Patent No. 4,615,974; and Bitter, U.S. Patent No. 4,977,092, which are incorporated herein by reference) and alcohol dehydrogenase genes. See also U.S. Patents Nos. 4,990,446; 5,063,154; 5,139,936 and 4,661,454, which are incorporated herein by reference. Transformation systems for other yeasts, including *Hansenula polymorpha*, *Schizosaccharomyces pombe*, *Kluyveromyces lactis*, *Kluyveromyces fragilis*, *Ustilago maydis*, *Pichia pastoris*, *Pichia methanolica*, *Pichia guillermondii* and *Candida maltosa* are known in the art. See, for example, Gleeson et al., J. Gen. Microbiol. 132:3459-3465, 1986 and Cregg, U.S. Patent No. 4,882,279. Aspergillus cells may be utilized according to the methods of McKnight et al., U.S. Patent No. 4,935,349, which is incorporated herein by reference. Methods for transforming *Acremonium chrysogenum* are disclosed by Sumino et al., U.S. Patent No. 5,162,228, which is incorporated herein by reference. Methods for transforming Neurospora are disclosed by Lambowitz, U.S. Patent No. 4,486,533, which is incorporated herein by reference.

Transformed or transfected host cells are cultured according to conventional procedures in a culture medium containing nutrients and other components required for the growth of the chosen host cells. A variety of suitable media, including defined media and complex media, are known in the art and generally include a carbon source, a nitrogen source, essential amino acids, vitamins and minerals. Media may also contain such components as growth factors or serum, as required. The growth medium will generally select for cells containing the exogenously added DNA by, for example, drug selection or deficiency in an essential nutrient which is complemented by the selectable marker carried on the expression vector or co-transfected into the host cell.

Within one aspect of the present invention, a novel receptor is produced by a cultured cell, and the cell is used to screen for ligands for the receptor, including the natural ligand, as well as agonists and antagonists of the natural ligand. To summarize this approach, a cDNA or gene encoding the receptor is combined with other genetic elements required for its expression (e.g., a transcription promoter), and the resulting expression vector is inserted into a host cell. Cells that express the DNA and produce functional receptor are selected and used within a variety of screening systems.

Mammalian cells suitable for use in expressing ZCytor2 receptors and transducing a receptor-mediated signal include cells that express a β-subunit, such as the human β_c subunit. In this regard it is generally preferred to employ a cell that is responsive to other cytokines that bind to receptors in the same subfamily, such as IL-3 or GM-CSF, because such cells will contain the requisite signal transduction pathway(s). It is also preferred to use a cell from the same species as the receptor to be expressed. Within a preferred embodiment, the cell is dependent upon an exogenously supplied hematopoietic growth factor for its proliferation.

Preferred cell lines of this type are the human TF-1 cell line (ATCC number CRL-2003) and the AML-193 cell line (ATCC number CRL-9589), which are GM-CSF-dependent human leukemic cell lines. In the alternative, suitable host cells can be engineered to produce a β-subunit (e.g., β_c) or other cellular component needed for the desired cellular response. For example, the murine cell line BaF3 (Palacios and Steinmetz, Cell 41: 727-734, 1985; Mathey-Prevot et al., Mol. Cell. Biol. 6: 4133-4135, 1986) or a baby hamster kidney (BHK) cell line can be transfected to express the human β_c subunit (also known as KH97) as well as a ZCytor2 receptor. The latter approach is advantageous because cell lines can be engineered to express receptor subunits from any species, thereby overcoming potential limitations arising from species specificity. In the alternative, species orthologs of the human receptor cDNA can be cloned and used within cell lines from the same species, such as a mouse cDNA in the BaF3 cell line. Cell lines that are dependent upon one hematopoietic growth factor, such as GM-CSF, can thus be engineered to become dependent upon a Zcytor2 ligand.

Cells expressing functional receptor are used within screening assays. A variety of suitable assays are known in the art. These assays are based on the detection of a biological response in a target cell. One such assay is a cell proliferation assay. Cells are cultured in the presence or absence of a test compound, and cell proliferation is detected by, for example, measuring incorporation of tritiated thymidine or by colorimetric assay based on the metabolic breakdown of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) (Mosman, J. Immunol. Meth. 65: 55-63, 1983). An alternative assay format uses cells that are further engineered to express a reporter gene. The reporter gene is linked to a promoter element that is responsive to the receptor-linked pathway, and the assay detects activation of transcription of the reporter gene. A preferred

promoter element in this regard is a serum response element, or SRE (see, e.g., Shaw et al., Cell 56:563-572, 1989). A preferred such reporter gene is a luciferase gene (de Wet et al., Mol. Cell. Biol. 7:725, 1987). Expression of the luciferase gene is detected by luminescence using methods known in the art (e.g., Baumgartner et al., J. Biol. Chem. 269:29094-29101, 1994; Schenborn and Goiffin, Promega Notes 41:11, 1993). Luciferase activity assay kits are commercially available from, for example, Promega Corp., Madison, WI. Target cell lines of this type can be used to screen libraries of chemicals, cell-conditioned culture media, fungal broths, soil samples, water samples, and the like. For example, a bank of cell-conditioned media samples can be assayed on a target cell to identify cells that produce ligand. Positive cells are then used to produce a cDNA library in a mammalian expression vector, which is divided into pools, transfected into host cells, and expressed. Media samples from the transfected cells are then assayed, with subsequent division of pools, re-transfection, subculturing, and re-assay of positive cells to isolate a cloned cDNA encoding the ligand.

A natural ligand for the ZCytor2 receptor can also be identified by mutagenizing a cell line expressing the receptor and culturing it under conditions that select for autocrine growth. See WIPO publication WO 95/21930. Within a typical procedure, BaF3 cells expressing ZCytor2 and human β_c are mutagenized, such as with 2-ethylmethanesulfonate (EMS). The cells are then allowed to recover in the presence of IL-3, then transferred to a culture medium lacking IL-3 and IL-4. Surviving cells are screened for the production of a ZCytor2 ligand, such as by adding soluble receptor to the culture medium or by assaying conditioned media on wild-type BaF3 cells and BaF3 cells expressing the receptor.

An additional screening approach provided by the present invention includes the use of hybrid receptor

polypeptides. These hybrid polypeptides fall into two general classes. Within the first class, the intracellular domain of Z-Cytor2, comprising approximately residues 364 to 380 of SEQ ID NO:2, is joined to the ligand-binding domain of a second receptor. It is preferred that the second receptor be a hematopoietic cytokine receptor, such as mpl receptor (Souyri et al., Cell 63: 1137-1147, 1990). The hybrid receptor will further comprise a transmembrane domain, which may be derived from either receptor. A DNA construct encoding the hybrid receptor is then inserted into a host cell. Cells expressing the hybrid receptor are cultured in the presence of a ligand for the binding domain and assayed for a response. This system provides a means for analyzing signal transduction mediated by ZCytor2 while using readily available ligands. This system can also be used to determine if particular cell lines are capable of responding to signals transduced by ZCytor2. A second class of hybrid receptor polypeptides comprise the extracellular (ligand-binding) domain of ZCytor2 (approximately residues 25 to 337 of SEQ ID NO:2) with an intracellular domain of a second receptor, preferably a hematopoietic cytokine receptor, and a transmembrane domain. Hybrid receptors of this second class are expressed in cells known to be capable of responding to signals transduced by the second receptor. Together, these two classes of hybrid receptors enable the use of a broad spectrum of cell types within receptor-based assay systems.

Cells found to express the ligand are then used to prepare a cDNA library from which the ligand-encoding cDNA can be isolated as disclosed above. The present invention thus provides, in addition to novel receptor polypeptides, methods for cloning polypeptide ligands for the receptors.

The tissue specificity of ZCytor2 expression suggests a role in spermatogenesis, a process that is

remarkably similar to the development of blood cells (hematopoiesis). Briefly, spermatogonia undergo a maturation process similar to the differentiation of hematopoietic stem cells. In both systems, the c-kit ligand is involved in the early stages of differentiation. In view of the tissue specificity observed for this receptor, agonists (including the natural ligand) and antagonists have enormous potential in both *in vitro* and *in vivo* applications. Compounds identified as receptor agonists are useful for stimulating proliferation and development of target cells *in vitro* and *in vivo*. For example, agonist compounds are useful as components of defined cell culture media, and may be used alone or in combination with other cytokines and hormones to replace serum that is commonly used in cell culture. Agonists are thus useful in specifically promoting the growth and/or development of testis-derived cells in culture. Agonists and antagonists may also prove useful in the study of spermatogenesis and infertility. Antagonists are useful as research reagents for characterizing sites of ligand-receptor interaction. *In vivo*, receptor agonists may find application in the treatment of male infertility. Antagonists of receptor function may be useful as male contraceptive agents.

Zcytor2 receptor antagonists and ligand-binding polypeptides may also be used to modulate immune functions by blocking the action of IL-13. Of particular interest in this regard is the limiting of unwanted immune responses, such as allergies and asthma. Local administration is preferred to avoid systemic immune suppression. Examples of local administration include topical application to the skin and inhalation. Suitable methods of formulation are known in the art.

Zcytor2 may also be used within diagnostic systems for the detection of circulating levels of ligand. Within a related embodiment, antibodies or other agents that specifically bind to Zcytor2 can be used to detect

circulating receptor polypeptides. Elevated or depressed levels of ligand or receptor polypeptides may be indicative of pathological conditions, including cancer.

ZCytor2 receptor polypeptides can be prepared by expressing a truncated DNA encoding residues 141 through 337 of a human Zcytor2 receptor (SEQ ID NO:2 or SEQ ID NO:4) or the corresponding region of a non-human receptor. Additional residues of the receptor may also be included, in particular amino-terminal residues between the predicted mature N-terminus (residue 25 of SEQ ID NO:2 or SEQ ID NO:4) and residue 141, and short C-terminal extensions. It is preferred that the extracellular domain polypeptides be prepared in a form substantially free of transmembrane and intracellular polypeptide segments. For example, the C-terminus of the receptor polypeptide may be at residue 338 or 339 of SEQ ID NO:2 or the corresponding region of an allelic variant or a non-human receptor. A preferred such polypeptide consists of residues 25 to 337 of SEQ ID NO:4. To direct the export of the receptor domain from the host cell, the receptor DNA is linked to a second DNA segment encoding a secretory peptide, such as a t-PA secretory peptide. To facilitate purification of the secreted receptor domain, a C-terminal extension, such as a poly-histidine tag, substance P, Flag™ peptide (Hopp et al., Biotechnology 6:1204-1210, 1988; available from Eastman Kodak Co., New Haven, CT) or another polypeptide or protein for which an antibody or other specific binding agent is available, can be fused to the receptor polypeptide.

In an alternative approach, a receptor extracellular domain can be expressed as a fusion with immunoglobulin heavy chain constant regions, typically an F_C fragment, which contains two constant region domains and a hinge region but lacks the variable region. Such fusions are typically secreted as multimeric molecules wherein the Fc portions are disulfide bonded to each other and two receptor polypeptides are arrayed in closed

proximity to each other. Fusions of this type can be used to affinity purify the cognate ligand from solution, as an *in vitro* assay tool, to block signals *in vitro* by specifically titrating out ligand, and as antagonists *in vivo* by administering them parenterally to bind circulating ligand and clear it from the circulation. To purify ligand, a Zcytor2-Ig chimera is added to a sample containing the ligand (e.g., cell-conditioned culture media or tissue extracts) under conditions that facilitate receptor-ligand binding (typically near-physiological temperature, pH, and ionic strength). The chimera-ligand complex is then separated by the mixture using protein A, which is immobilized on a solid support (e.g., insoluble resin beads). The ligand is then eluted using conventional chemical techniques, such as with a salt or pH gradient. In the alternative, the chimera itself can be bound to a solid support, with binding and elution carried out as above. The chimeras may be used *in vivo* to induce infertility. Chimeras with high binding affinity are administered parenterally (e.g., by intramuscular, subcutaneous or intravenous injection). Circulating molecules bind ligand and are cleared from circulation by normal physiological processes. For use in assays, the chimeras are bound to a support via the F_c region and used in an ELISA format.

A preferred assay system employing a ligand-binding receptor fragment uses a commercially available biosensor instrument (BIAcore™, Pharmacia Biosensor, Piscataway, NJ), wherein the receptor fragment is immobilized onto the surface of a receptor chip. Use of this instrument is disclosed by Karlsson, J. Immunol. Methods 145:229-240, 1991 and Cunningham and Wells, J. Mol. Biol. 234:554-563, 1993. A receptor fragment is covalently attached, using amine or sulfhydryl chemistry, to dextran fibers that are attached to gold film within the flow cell. A test sample is passed through the cell. If ligand is present in the sample, it will bind to the

immobilized receptor polypeptide, causing a change in the refractive index of the medium, which is detected as a change in surface plasmon resonance of the gold film. This system allows the determination of on- and off-rates, from which binding affinity can be calculated, and assessment of stoichiometry of binding.

Ligand-binding receptor polypeptides can also be used within other assay systems known in the art. Such systems include Scatchard analysis for determination of binding affinity (see, Scatchard, Ann. NY Acad. Sci. 51: 660-672, 1949) and calorimetric assays (Cunningham et al., Science 253:545-548, 1991; Cunningham et al., Science 254:821-825, 1991).

A receptor ligand-binding polypeptide can also be used for purification of ligand. The receptor polypeptide is immobilized on a solid support, such as beads of agarose, cross-linked agarose, glass, cellulosic resins, silica-based resins, polystyrene, cross-linked polyacrylamide, or like materials that are stable under the conditions of use. Methods for linking polypeptides to solid supports are known in the art, and include amine chemistry, cyanogen bromide activation, N-hydroxysuccinimide activation, epoxide activation, sulfhydryl activation, and hydrazide activation. The resulting media will generally be configured in the form of a column, and fluids containing ligand are passed through the column one or more times to allow ligand to bind to the receptor polypeptide. The ligand is then eluted using changes in salt concentration or pH to disrupt ligand-receptor binding.

Zcytor2 polypeptides can also be used to prepare antibodies that specifically bind to Zcytor2 polypeptides. As used herein, the term "antibodies" includes polyclonal antibodies, monoclonal antibodies, antigen-binding fragments thereof such as $F(ab')_2$ and Fab fragments, and the like, including genetically engineered antibodies. Antibodies are defined to be specifically binding if they

bind to a Zcytor2 polypeptide with a K_a of greater than or equal to 10^7/M. The affinity of a monoclonal antibody can be readily determined by one of ordinary skill in the art (see, for example, Scatchard, ibid.).

Methods for preparing polyclonal and monoclonal antibodies are well known in the art (see for example, Sambrook et al., Molecular Cloning: A Laboratory Manual, Second Edition, Cold Spring Harbor, NY, 1989; and Hurrell, J. G. R., Ed., Monoclonal Hybridoma Antibodies: Techniques and Applications, CRC Press, Inc., Boca Raton, FL, 1982, which are incorporated herein by reference). As would be evident to one of ordinary skill in the art, polyclonal antibodies can be generated from a variety of warm-blooded animals such as horses, cows, goats, sheep, dogs, chickens, rabbits, mice, and rats. The immunogenicity of a Zcytor2 polypeptide may be increased through the use of an adjuvant such as Freund's complete or incomplete adjuvant. A variety of assays known to those skilled in the art can be utilized to detect antibodies which specifically bind to Zcytor2 polypeptides. Exemplary assays are described in detail in Antibodies: A Laboratory Manual, Harlow and Lane (Eds.), Cold Spring Harbor Laboratory Press, 1988. Representative examples of such assays include: concurrent immunoelectrophoresis, radio-immunoassays, radio-immunoprecipitations, enzyme-linked immunosorbent assays (ELISA), dot blot assays, inhibition or competition assays, and sandwich assays.

Antibodies to Zcytor2 are may be used for tagging cells that express the receptor, for affinity purification, within diagnostic assays for determining circulating levels of soluble receptor polypeptides, and as antagonists to block ligand binding and signal transduction *in vitro* and *in vivo*.

The invention is further illustrated by the following non-limiting examples.

Example 1

A cDNA library was prepared from human placental poly A^+ RNA provided as a control in a Marathon™ cDNA Amplification Kit (Clontech, Palo Alto, CA) using the protocol provided by the manufacturer. This cDNA was used as template in polymerase chain reactions to generate DNA encoding human Zcytor2.

Primers were designed from the sequences of two expressed sequence tags (ESTs) in a DNA sequence database. Analysis of the EST sequences suggested that they represented the 5' and 3' ends of a cDNA encoding a cytokine receptor. One pair of primers, designated ZG9801 (SEQ ID NO:8) and ZG9941 (SEQ ID NO:9), were designed to be used in a 5' RACE (rapid amplification of cDNA ends) reaction. A second pair, designated ZG9803 (SEQ ID NO:10) and ZG9937 (SEQ ID NO:11), were designed to be used in a 3' RACE reaction. A third pair of primers, designated ZG9800 (SEQ ID NO:12) and ZG9802 (SEQ ID NO:13), were designed to amplify the region spanning the two ESTs. A fourth pair of primers, AP1 (SEQ ID NO:14) and AP2 (SEQ ID NO:15), were supplied with the amplification kit or synthesized.

PCR amplification was carried out according to the instruction manual supplied with the kit, with certain modifications to the protocol. For the 5' and 3' RACE reactions, fifty pmol of each primer was used in each reaction. Each cDNA template was initially amplified using the appropriate gene-specific primer (ZG9801 or ZG9803) for 10 cycles. Primer AP1 was then added, and the reaction was continued for 25 cycles. The reaction mixture was incubated in a Hybaid OmniGene Temperature Cycling System (National Labnet Co., Woodbridge, NY) for 1 minute at 95°C, then for 10 cycles of 60°C, 30 seconds; 72° C, 2 minutes; 95°C, 30 seconds. The mixture was held at 60 °C, and 50 pmol of primer AP1 was added, and the reaction was continued for 25 cycles of 60°C, 30 seconds; 72°C, 2 minutes; 95°C, 30 seconds; followed by a 7 minute

incubation at 72°C. The internal fragment was amplified under the same conditions using gene-specific primers (9800 and 9802), but AP1 was omitted. Reaction products were analyzed by electrophoresis on a 1% agarose gel. A discreet band was obtained for the internal fragment. The 5' and 3' RACE products were smears on the gel.

The 5' and 3' RACE products were purified using a PCR purification kit (Qiagen Inc., Chatsworth, CA) and used in nested PCR reactions. Each template was combined with 50 pmol of the appropriate specific primer (ZG9941 or ZG9937) and 50 pmol of primer AP2. Reactions were run for 30 cycles of 95°C, 1 minute; 60°C, 30 seconds; 72°C, 3.5 minutes; then incubated at 72°C for 7 minutes. The reaction products were analyzed by electrophoresis on a 1% agarose gel. One discreet band was obtained for each reaction.

The 5' and 3' products from the nested PCR reactions and the internal fragment from the initial Marathon™ PCR reaction were gel purified using a Qiagen Gel Extraction Kit.

The internal fragment was subcloned using a Stratagene (La Jolla, CA) pCR-Script™ SK(+) Cloning Kit according to the manufacturer's instructions, with 10 µl H_2O added to each reaction. The ligated DNA was then purified using CENTRI-SEP columns (Princeton Separations, Adelphia, NJ) to increase the efficiency of transformation. The resulting vector was used to transform *E. coli* ElectroMAX DH10B™ cells (Gibco BRL, Gaithersburg, MD) by electroporation.

Colonies were screened by PCR using gene-specific primers. Individual white colonies representing recombinants were picked and added to microcentrifuge tubes by swirling the toothpick with the colony on it in a tube containing 19.5 µl H_2O, 2.5 µl 10x Taq polymerase buffer (Boehringer Mannheim, Indianapolis, IN), 0.5 µl 10 mM dNTPs, 1.0 µl ZG9800 (SEQ ID NO:12) (20 pmol/µl), 10 µl ZG9802 (SEQ ID NO:13) (20 pmol/µl), and 0.5 µl Taq

polymerase. Cells were streaked out on a master plate to use for starting cultures. Amplification reactions were incubated at 96°C for 45 seconds to lyse the bacteria and expose the plasmid DNA, then run for 25 cycles of 96°C, 45 seconds; 55°C, 45 seconds; 72°C, 2 minutes to amplify cloned inserts. Products were analyzed by electrophoresis on a 1% agarose gel. One clone was identified as positive, and a plasmid template was prepared for sequencing using a QIAwell™ 8 Plasmid Kit (Qiagen Inc.).

The 5' RACE product, the 3' RACE product, the internal fragment and the internal fragment subclone were sequenced on an Applied Biosystems™ model 373 DNA sequencer (Perkin-Elmer Corporation, Norwalk, CT) using either an AmpliTaq® DyeDeoxy™ Terminator Cycle Sequencing Kit (Perkin-Elmer Corp.) or an ABI PRISM™ Dye Terminator Cycle Sequencing Core Kit (Perkin-Elmer Corp.). Oligonucleotides used in the PCR reactions were used as sequencing primers. In addition, primers ZG9850 (SEQ ID NO:16), ZG9851 (SEQ ID NO:17), ZG9852 (SEQ ID NO:18) and ZG9919 (SEQ ID NO:19) were used. Sequencing reactions were carried out in a Hybaid OmniGene Temperature Cycling System. Sequencher™ 3.0 sequence analysis software (Gene Codes Corporation, Ann Arbor, MI) was used for data analysis. Although the internal fragment subclone contained the entire coding sequence for the receptor, a composite sequence was constructed from all templates to include additional 5' and 3' untranslated sequence from the RACE products that was not present in the internal subclone. The full sequence is dislosed in SEQ ID NO:1.

A human cDNA was isolated by PCR using oligonucleotide primers specific for the gene sequence and containing restriction sites for subsequent manipulation of the DNA. Specific DNA was amplified from a human testis cDNA library using primers ZG10317 (SEQ ID NO:20) and ZG10319 (SEQ ID NO:21). 10 ng of template DNA was combined with 20 pmol of each primer, 5 µl of 10X buffer (Takara Shuzo Co., Ltd., Otsu, Shiga, Japan), 1 µl of

ExTaq DNA polymerase (Takara Shuzo Co., Ltd.), and 200 µM dNTPs. The reaction was run for 30 cycles of 95°C, 30 seconds; 55°C, 30 seconds, and 68°C, 2 minutes; then incubated at 68°C for 10 minutes. A fragment of approximately 1200 bp was recovered using a Wizard™ PCR Preps Purification System (Promega Corp., Madison, WI), cleaved with Xho I and Xba I, and a 1200 bp fragment was recovered by precipitation with ethanol.

The 1200 bp fragment was ligated into pHZ200, a vector comprising the mouse metallothionein-1 promoter, the bacteriophage T7 promoter flanked by multiple cloning banks containing unique restriction sites for insertion of coding sequences, the human growth hormone terminator, the bacteriophage T7 terminator, an *E. coli* origin of replication, a bacterial beta lactamase gene, and a mammalian selectable marker expression unit comprising the SV40 promoter and origin, a DHFR gene, and the SV40 transcription terminator. Plasmid pHZ200 was cleaved with Sal I and Xba I and was ligated to the Zcytor2 fragment.

The sequence of the human testis cDNA clone and the deduced amino acid sequence are shown in SEQ ID NO:3 and SEQ ID NO:4, respectively. The deduced amino acid sequence differs from that shown in SEQ ID NO:2 at residues 65, 180, and 259.

Example 2

Human Multiple Tissue Northern Blots (Human I, Human II, and Human III from Clontech) were probed to determine the tissue distribution of ZCytor2 expression. A probe was prepared by PCR. Single stranded DNA was prepared from K-562 mRNA (obtained from Clontech) using a RT-PCR kit (Stratagene Cloning Systems, La Jolla, CA) for use as template. 10 ng of template DNA was combined with 20 pmol of each of primers ZG9820 (SEQ ID NO:22) and ZG9806 (SEQ ID NO:23), 5 µl of 10X buffer (Clontech), 1 µl of KlenTaq DNA polymerase (Clontech), and 200 µM dNTPs. The reaction was run for 30 cycles of 95°C, 30 seconds;

55°C, 30 seconds, and 68°C, 2 minutes; then incubated at 68°C for 10 minutes. The resulting DNA was purified by gel electrophoresis and ligated into pGEM®A/T (Promega Corp.). The resulting plasmid was used as a PCR template to generate the probe using the same reaction conditions described above for the K-562 template. DNA was purified by gel electrophoresis and labeled with ^{32}P by random priming. The blots were prehybridized in ExpressHyb™ hybridization solution (Clontech) at 65°C for 1-6 hours, then hybridized in ExpressHyb™ solution containing 2 x 10^6 cpm/ml of probe at 65°C for from 1.5 hour to overnight. After hybridization the blots were washed at 50°C in 0.1X SSC, 0.1% SDS. A transcript of approximately 1.5 kb was seen only in testis.

Example 3

A cDNA encoding a soluble human ZCytor2 receptor polypeptide was prepared by PCR. Human cDNA was prepared from a human testis cDNA library. DNA was amplified by PCR using 10 pmol each of oligonucleotide primers ZG10320 (SEQ ID NO:24) and ZG10318 (SEQ ID NO:25). 10 ng of template DNA was combined with 20 pmol of each primer, 5 µl of 10X buffer (Takara Shuzo Co., Ltd.), 1 µl of Taq DNA polymerase (Boehringer Mannheim), and 200 µM dNTPs. The reaction was run for 30 cycles of 95°C, 30 seconds; 55°C, 30 seconds, and 68°C, 2 minutes; then incubated at 68°C for ten minutes. PCR products were separated by electrophoresis on a low melting point agarose gel (Boehringer Mannheim) and purified using a Wizard™ PCR Preps Purification System (Promega Corp.). The fragment was inserted into plasmid HSRT9 that had been cleaved with Bgl II and Xho I. HSRT9 is a mammalian cell expression vector derived from pHZ200 that contains a tissue plasminogen activator (t-PA) secretory signal sequence and a sequence encoding a C-terminal polyhistidine tag downstream of the MT-1 promoter. The resulting construct

encoded a t-PA secretory peptide, human Zcytor2 residues 25--339 (SEQ ID NO:4), and a polyhistidine tag.

The soluble receptor expression vector is transfected into BHK 570 cells (ATCC No. CRL-10314) by liposome-mediated transfection (LIPOFECTAMINE™ Reagent, Life Technologies, Gaithersburg, MD). Transfectants are cultured in the presence of methotrexate to select and amplify the transfected DNA. Soluble receptor polypeptide is recovered from conditioned culture media on nickel affinity purification columns (e.g., Talon spin columns from Clontech Laboratories). Columns are washed at neutral pH, and protein is eluted using a decreasing pH gradient or an imidazole gradient. Receptor monomers elute at about pH 6.0-6.3 of 50 mM imidazole, and receptor dimers elute at about pH 5.0-5.3 or 100 mM imidazole. In the alternative, batch purification can be employed.

Example 4

A cDNA library was prepared from a non-human primate. Testis tissue was obtained from a 13-year-old Celebus macaque. Total RNA was prepared from the tissue by the CsCl method (Chirgwin et al., Biochemistry 18:52-94, 1979). Poly(A)$^+$ RNA was prepared from the total RNA by oligo(dT) cellulose chromatography (Aviv and Leder, Proc. Natl. Acad. Sci. USA 69:1408-1412, 1972). Double-stranded DNA was prepared from 1 µg of mRNA using a commercially available kit (Clontech Marathon™ cDNA amplification kit).

The macaque cDNA was amplified by PCR using a standard adapter-primer and primers derived from the human receptor cDNA sequence. Individual PCR mixtures (50 µl total volume) contained 5 µl template DNA, 5 µl 10X buffer (Clontech), 200 µM dNTPs (Perkin Elmer, CITY), 1 µl each of 10 pmol/µl primer AP1 (Clontech) and one of the primers (20 pmol/µl) shown in Table 4, and 1 µl of Klentaq DNA polymerase (Clontech). The reactions were run for 3 cycles of 94°C, 30 seconds; 65°C, 30 seconds; 68°C, 30

seconds; 3 cycles of 94°C, 30 seconds; 60°C, 30 seconds; 68 °C, 30 seconds; 3 cycles of 94°C, 30 seconds; 55°C, 30 seconds; 68°C, 30 seconds; and 30 cycles of 94°C, 30 seconds; 50°, 30 seconds; 68°C, 30 seconds; followed by a 68°C incubation for 10 minutes.

Table 4

Reaction No.	Primer No.	Primer SEQ ID NO.
1	9800	12
2	9820	22
3	9941	9
4	9801	8
5	9882	26
6	10082	27
7	9850	16
8	9919	16
9	10083	28
10	9803	10
11	10081	29
12	9881	30
13	9937	11
14	9806	23
15	9802	13

PCR products were electrophoresed on an agarose gel. The gel was stained with ethidium bromide and viewed under ultraviolet light. Bands from reactions amplified with primers 9800 and 9802 were of the expected size.

A second set of PCR reactions was run using the macaque cDNA (1:250 dilution) or first round PCR products from reactions 1, 2, 14 or 15 (Table 4) as templates. the first round PCR products were purified using a Wizard™ PCR Preps Purification System (Promega Corp.) prior to use. 5 µl of template DNA was combined with other components as shown in Table 5. 1 µl of Klentaq DNA polymerase (Clontech) was added to each mixture. Reaction conditions

were as specified above. Reaction products were electrophoresed on an agarose gel, stained with ethidium bromide, and visualized under UV light.

Table 5

Rxn. No.	Template	10x Buffer	dNTPs	Primer 1	Primer 2	H_2O
1	macaque	5 µl	0.5 µl	--	--	36.5 µl
2	macaque	5 µl	0.5 µl	9800	--	36.5 µl
3	macaque	5 µl	0.5 µl	9802	--	36.5 µl
4	macaque	5 µl	0.5 µl	9800	AP1	36.5 µl
5	macaque	5 µl	0.5 µl	9802	AP1	36.5 µl
6	macaque	5 µl	0.5 µl	AP1	--	36.5 µl
7	macaque	5 µl	0.5 µl	AP1	3'GP3DH	36.5 µl
8	macaque	5 µl	0.5 µl	AP1	5'GP3DH	36.5 µl
9	#14	5 µl	0.5 µl	AP1	9806	36.5 µl
10	#15	5 µl	0.5 µl	AP1	9802	36.5 µl
11	#1	5 µl	0.5 µl	AP1	9800	36.5 µl
12	#2	5 µl	0.5 µl	AP1	9820	36.5 µl

Partial DNA and deduced amino acid sequences of macaque Zcytor2 cDNA are shown in SEQ ID NO:6 and SEQ ID NO:7. Alignment of the human and partial macaque sequences showed an amino acid sequence identity of 92% and a nucleotide sequence identity of 96%.

Example 5

An expression vector encoding a human Zcytor2-IgG fusion protein was constructed. The fusion comprised the extracellular domain of Zcytor2 fused at its C-terminus (residue 339 of SEQ ID NO:4) to the hinge region of the Fc portion of an $IgG_{\gamma 1}$ (Ellison et al., Nuc. Acids Res. 10:4071-4079, 1982). The hinge region was modified to replace a cysteine residue with serine to avoid unpaired cysteines upon dimerization of the fusion

protein. A human t-PA secretory peptide was used to direct secretion of the fusion.

A human Zcytor2 DNA was prepared from a testis cDNA library by PCR using oligonucleotide primers ZG10320 (SEQ ID NO:24) and ZG10389 (SEQ ID NO:31). Twenty pmol of each primer was combined with 1 µl (10 ng) of template DNA, 10 µl of 2.5 mM dNTPs (Perkin-Elmer Corp.), 10 µl of 10X buffer (Klentaq PCR buffer, Clontech), 2 µl of Klentaq DNA polymerase (Clontech), and 70.8 µl H_2O. The reaction was run for 35 cycles of 94°C, 1 minute; 55°C, 1 minute; and 72°C, 2 minutes; followed by a 7 minute incubation at 72°C. The reaction products were extracted with phenol/$CHCl_3$, precipitated with ethanol, and digested with BglII. The DNA was electrophoresed on a agarose gel, and a 941 bp fragment was electrophoretically eluted from a gel slice, purified by phenol/$CHCl_3$ extraction, and precipitated with ethanol.

A human $IgG_{\gamma 1}$ clone was isolated from a human fetal liver cDNA library (Clontech) by PCR using oligonucleotide primers ZG10314 (SEQ ID NO:32) and ZG10315 (SEQ ID NO:33). The former primer introduced a BglII site into the hinge region (changing the third residue of the hinge region from Lys to Arg) and replaced the fifth residue of the hinge region (Cys) with Ser. PCR was carried out essentially as described above for the Zcytor2 extracellular domain sequence. The DNA was digested with EcoRI and XbaI, and a 0.7 kb fragment was recovered by agarose gel electrophoresis, electroelution, phenol/$CHCl_3$ extraction, and ethanol precipitation. The IgG-encoding fragment and an XbaI-EcoRI linker were ligated into Zem229R (ATCC Accession No. 69447) that had been digested with EcoRI and treated with calf intestinal phosphatase. The resulting plasmid was digested with BglII and XbaI, and a 950 bp fragment was recovered by agarose gel electrophoresis, electroelution, phenol/$CHCl_3$ extraction, and ethanol precipitation.

To construct an expression vector for the Zcytor2-IgG fusion, a Zem229R vector containing a human t-PA secretory signal sequence joned to a human thrombopoietin sequence (disclosed in copending, commonly assigned U.S. Patent Application Serial No. 08/347,029) was cleaved with BglII and XbaI. The fragment comprising the vector and t-PA secretory signal sequence was recovered and ligated to the IgG fragment. The Zcytor2 fragment was then ligated into this construct at the BglII site. The resulting plasmid was screened for the desired insert orientation. A plasmid with the desired orientation was designated h-Zcytor-2/IgG #709. Sequence analysis revealed a PCR-generated substitution resulting in an alanine codon instead of a valine codon at position 308 of SEQ ID NO:3.

Plasmid h-Zcytor-2/IgG was transfected into BHK-570 cells by liposome-mediated transfection (LIPOFECTAMINE™ Reagent, Life Technologies, Gaithersburg, MD). Transfectants were cultured in medium containing 1 µM methotrexate for 10 days.

Example 6

The binding of ^{125}I-IL-13 to wild-type and Zcytor2-transfected BHK, TF-1, and BaF3 cells was determined. BHK cells were assayed in 6-well culture plates. TF-1 and BaF3 cells were assayed in microcentrifuge tubes. Cells were combined with 500 µl of either solution A (15 ml of binding buffer [RPMI containing 20 mM Tris pH7.4, 0.05% NaN_3, and 3 mg/ml BSA] plus 263 µl of ^{125}I-IL-13 [5.7 x 10^7 cpm/ml]) or solution B (solution A containing 15 µl of cold 25 µg/ml IL-13). After a 2-hour incubation, cells were washed three times with 500 µl binding buffer and lysed in 500 µl of 400 mM NaOH. Lysates were transferred to tubes for gamma counting. BHK cells transfected to express Zcytor2 were found to specifically bind significant amounts of IL-13.

In further experiments, binding of labeled IL-13 was found to be inhibited by IL-13 but not by IL-4.

Saturation binding analysis indicated that Zcytor2 expressed in BHK cells bound ^{125}I-IL-13 with a kd of 590 ± 359 pM.

To determine if a soluble Zcytor2-IgG fusion could specifically bind IL-13, 1 µg of purified fusion protein was incubated in 200 µl of binding buffer containing 1 nM ^{125}I-IL-13 ± 100 nM unlabeled IL-13 or IL-4. After two hours at room temperature with mixing, 25 µl of protein A-Sepharose was added, and the mixtures were incubated for an additional hour. The Sepharose was washed three times and collected by centrifugation. Bound ^{125}I-IL-13 was determined by gamma counting. The fusion protein was found to bind significant amounts of labeled IL-13, which was blocked by excess unlabeled IL-13 but not by IL-4.

Binding of labeled IL-13 by BHK/Zcytor2 cells was measured in the presence and absence of the soluble Zcytor2-IgG fusion (0.005 - 5 ng/ml) or unlabeled IL-13. Binding was assayed essentially as described above. Both IL-13 and the fusion protein were found to inhibit binding of labeled IL-13 to the cells.

From the foregoing, it will be appreciated that, although specific embodiments of the invention have been described herein for purposes of illustration, various modifications may be made without deviating from the spirit and scope of the invention. Accordingly, the invention is not limited except as by the appended claims.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: ZymoGenetics, Inc.
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Seattle
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98102

(ii) TITLE OF INVENTION: Testis-Specific Receptor

(iii) NUMBER OF SEQUENCES: 33

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: ZymoGenetics, Inc.
(B) STREET: 1201 Eastlake Avenue East
(C) CITY: Seattle
(D) STATE: WA
(E) COUNTRY: USA
(F) ZIP: 98102

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Parker, Gary E.
(B) REGISTRATION NUMBER: 31,648
(C) REFERENCE/DOCKET NUMBER: 95-33

(ix) TELECOMMUNICATION INFORMATION:
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(B) TELEFAX: 206-442-6678

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1289 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 49..1191

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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CCCCCCGCCC GGGAGAGAGG CAATATCAAG GTTTTAAATC TCGGAGAA ATG GCT TTC      57
                                                      Met Ala Phe
                                                        1

GTT TGC TTG GCT ATC GGA TGC TTA TAT ACC TTT CTG ATA AGC ACA ACA      105
Val Cys Leu Ala Ile Gly Cys Leu Tyr Thr Phe Leu Ile Ser Thr Thr
  5                  10                  15

TTT GGC TGT ACT TCA TCT TCA GAC ACC GAG ATA AAA GTT AAC CCT CCT      153
Phe Gly Cys Thr Ser Ser Ser Asp Thr Glu Ile Lys Val Asn Pro Pro
 20                  25                  30                  35

CAG GAT TTT GAG ATA GTG GAT CCC GGA TAC TTA GGT TAT CTC TAT TTG      201
Gln Asp Phe Glu Ile Val Asp Pro Gly Tyr Leu Gly Tyr Leu Tyr Leu
                 40                  45                  50

CAA TGG CAA CCC CCA CTG TCT CTG GAT CAT TTT AAG GAA TGC ACA GTG      249
Gln Trp Gln Pro Pro Leu Ser Leu Asp His Phe Lys Glu Cys Thr Val
             55                  60                  65

GAA TAT GAA CTA AAA TAC CGA AAC ATT GGT AGT GAA ACA TGG AAG ACC      297
Glu Tyr Glu Leu Lys Tyr Arg Asn Ile Gly Ser Glu Thr Trp Lys Thr
         70                  75                  80

ATC ATT ACT AAG AAT CTA CAT TAC AAA GAT GGG TTT GAT CTT AAC AAG      345
Ile Ile Thr Lys Asn Leu His Tyr Lys Asp Gly Phe Asp Leu Asn Lys
  85                  90                  95
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GGC ATT GAA GCG AAG ATA CAC ACG CTT TTA CCA TGG CAA TGC ACA AAT          393
Gly Ile Glu Ala Lys Ile His Thr Leu Leu Pro Trp Gln Cys Thr Asn
100                 105                 110                 115

GGA TCA GAA GTT CAA AGT TCC TGG GCA GAA ACT ACT TAT TGG ATA TCA          441
Gly Ser Glu Val Gln Ser Ser Trp Ala Glu Thr Thr Tyr Trp Ile Ser
                120                 125                 130

CCA CAA GGA ATT CCA GAA ACT AAA GTT CAG GAT ATG GAT TGC GTA TAT          489
Pro Gln Gly Ile Pro Glu Thr Lys Val Gln Asp Met Asp Cys Val Tyr
            135                 140                 145

TAC AAT TGG CAA TAT TTA CTC TGT TCT TGG AAA CCT GGC ATA GGT GTA          537
Tyr Asn Trp Gln Tyr Leu Leu Cys Ser Trp Lys Pro Gly Ile Gly Val
        150                 155                 160

CTT CTT GAT ACC AAT TAC AAC TTG TTT TAC TGG TAT GAG GGC TTG GAT          585
Leu Leu Asp Thr Asn Tyr Asn Leu Phe Tyr Trp Tyr Glu Gly Leu Asp
    165                 170                 175

CAT GCA TTA CAG TGT GTT GAT TAC ATC AAG GCT GAT GGA CAA AAT ATA          633
His Ala Leu Gln Cys Val Asp Tyr Ile Lys Ala Asp Gly Gln Asn Ile
180                 185                 190                 195

GGA TGC AGA TTT CCC TAT TTG GAG GCA TCA GAC TAT AAA GAT TTC TAT          681
Gly Cys Arg Phe Pro Tyr Leu Glu Ala Ser Asp Tyr Lys Asp Phe Tyr
                200                 205                 210

ATT TGT GTT AAT GGA TCA TCA GAG AAC AAG CCT ATC AGA TCC AGT TAT          729
Ile Cys Val Asn Gly Ser Ser Glu Asn Lys Pro Ile Arg Ser Ser Tyr
            215                 220                 225

TTC ACT TTT CAG CTT CAA AAT ATA GTT AAA CCT TTG CCG CCA GTC TAT          777
Phe Thr Phe Gln Leu Gln Asn Ile Val Lys Pro Leu Pro Pro Val Tyr
        230                 235                 240

CTT ACT TTT ACT CGG GAG AGT TCA TGT GAA ATT AAG CTG AAA TGG AGC          825
Leu Thr Phe Thr Arg Glu Ser Ser Cys Glu Ile Lys Leu Lys Trp Ser
    245                 250                 255

ATA CCT TTG GGA CCT ATT CCA GCA AGG TGT TTT GAT TAT GAA ATT GAG          873
Ile Pro Leu Gly Pro Ile Pro Ala Arg Cys Phe Asp Tyr Glu Ile Glu
260                 265                 270                 275
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ATC AGA GAA GAT GAT ACT ACC TTG GTG ACT GCT ACA GTT GAA AAT GAA          921
Ile Arg Glu Asp Asp Thr Thr Leu Val Thr Ala Thr Val Glu Asn Glu
                280                 285                 290

ACA TAC ACC TTG AAA ACA ACA AAT GAA ACC CGA CAA TTA TGC TTT GTA          969
Thr Tyr Thr Leu Lys Thr Thr Asn Glu Thr Arg Gln Leu Cys Phe Val
            295                 300                 305

GTA AGA AGC AAA GTG AAT ATT TAT TGC TCA GAT GAC GGA ATT TGG AGT          1017
Val Arg Ser Lys Val Asn Ile Tyr Cys Ser Asp Asp Gly Ile Trp Ser
        310                 315                 320

GAG TGG AGT GAT AAA CAA TGC TGG GAA GGT GAA GAC CTA TCG AAG AAA          1065
Glu Trp Ser Asp Lys Gln Cys Trp Glu Gly Glu Asp Leu Ser Lys Lys
    325                 330                 335

ACT TTG CTA CGT TTC TGG CTA CCA TTT GGT TTC ATC TTA ATA TTA GTT          1113
Thr Leu Leu Arg Phe Trp Leu Pro Phe Gly Phe Ile Leu Ile Leu Val
340                 345                 350                 355

ATA TTT GTA ACC GGT CTG CTT TTG CGT AAG CCA AAC ACC TAC CCA AAA          1161
Ile Phe Val Thr Gly Leu Leu Leu Arg Lys Pro Asn Thr Tyr Pro Lys
                360                 365                 370

ATG ATT CCA GAA TTT TTC TGT GAT ACA TGAAGACTTT CCATATCAAG                1208
Met Ile Pro Glu Phe Phe Cys Asp Thr
            375                 380

AGACATGGTA TTGACTCAAC AGTTTCCAGT CATGGCCAAA TGTTCAATAT GAGTCTCAAT       1268

AAACTGAATT TTTCTTGCGA A                                                 1289
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(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 380 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met Ala Phe Val Cys Leu Ala Ile Gly Cys Leu Tyr Thr Phe Leu Ile
1 5 10 15

Ser Thr Thr Phe Gly Cys Thr Ser Ser Ser Asp Thr Glu Ile Lys Val
20 25 30

Asn Pro Pro Gln Asp Phe Glu Ile Val Asp Pro Gly Tyr Leu Gly Tyr
35 40 45

Leu Tyr Leu Gln Trp Gln Pro Pro Leu Ser Leu Asp His Phe Lys Glu
50 55 60

Cys Thr Val Glu Tyr Glu Leu Lys Tyr Arg Asn Ile Gly Ser Glu Thr
65 70 75 80

Trp Lys Thr Ile Ile Thr Lys Asn Leu His Tyr Lys Asp Gly Phe Asp
85 90 95

Leu Asn Lys Gly Ile Glu Ala Lys Ile His Thr Leu Leu Pro Trp Gln
100 105 110

Cys Thr Asn Gly Ser Glu Val Gln Ser Ser Trp Ala Glu Thr Thr Tyr
115 120 125

Trp Ile Ser Pro Gln Gly Ile Pro Glu Thr Lys Val Gln Asp Met Asp
130 135 140

Cys Val Tyr Tyr Asn Trp Gln Tyr Leu Leu Cys Ser Trp Lys Pro Gly
145 150 155 160

Ile Gly Val Leu Leu Asp Thr Asn Tyr Asn Leu Phe Tyr Trp Tyr Glu
165 170 175

Gly Leu Asp His Ala Leu Gln Cys Val Asp Tyr Ile Lys Ala Asp Gly
180 185 190

Gln Asn Ile Gly Cys Arg Phe Pro Tyr Leu Glu Ala Ser Asp Tyr Lys
195 200 205

Asp Phe Tyr Ile Cys Val Asn Gly Ser Ser Glu Asn Lys Pro Ile Arg
210 215 220

Ser Ser Tyr Phe Thr Phe Gln Leu Gln Asn Ile Val Lys Pro Leu Pro
225 230 235 240

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Pro Val Tyr Leu Thr Phe Thr Arg Glu Ser Ser Cys Glu Ile Lys Leu
                245                 250                 255

Lys Trp Ser Ile Pro Leu Gly Pro Ile Pro Ala Arg Cys Phe Asp Tyr
            260                 265                 270

Glu Ile Glu Ile Arg Glu Asp Asp Thr Thr Leu Val Thr Ala Thr Val
        275                 280                 285

Glu Asn Glu Thr Tyr Thr Leu Lys Thr Thr Asn Glu Thr Arg Gln Leu
    290                 295                 300

Cys Phe Val Val Arg Ser Lys Val Asn Ile Tyr Cys Ser Asp Asp Gly
305                 310                 315                 320

Ile Trp Ser Glu Trp Ser Asp Lys Gln Cys Trp Glu Gly Glu Asp Leu
                325                 330                 335

Ser Lys Lys Thr Leu Leu Arg Phe Trp Leu Pro Phe Gly Phe Ile Leu
            340                 345                 350

Ile Leu Val Ile Phe Val Thr Gly Leu Leu Leu Arg Lys Pro Asn Thr
        355                 360                 365

Tyr Pro Lys Met Ile Pro Glu Phe Phe Cys Asp Thr
    370                 375                 380
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(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1167 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 10..1152

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

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GATCCGCCC ATG GCT TTC GTT TGC TTG GCT ATC GGA TGC TTA TAT ACC          48
          Met Ala Phe Val Cys Leu Ala Ile Gly Cys Leu Tyr Thr
            1               5                  10

TTT CTG ATA AGC ACA ACA TTT GGC TGT ACT TCA TCT TCA GAC ACC GAG        96
Phe Leu Ile Ser Thr Thr Phe Gly Cys Thr Ser Ser Ser Asp Thr Glu
     15                  20                  25

ATA AAA GTT AAC CCT CCT CAG GAT TTT GAG ATA GTG GAT CCC GGA TAC       144
Ile Lys Val Asn Pro Pro Gln Asp Phe Glu Ile Val Asp Pro Gly Tyr
 30                  35                  40                  45

TTA GGT TAT CTC TAT TTG CAA TGG CAA CCC CCA CTG TCT CTG GAT CAT       192
Leu Gly Tyr Leu Tyr Leu Gln Trp Gln Pro Pro Leu Ser Leu Asp His
                 50                  55                  60

TTT AAG GAA TAC ACA GTG GAA TAT GAA CTA AAA TAC CGA AAC ATT GGT       240
Phe Lys Glu Tyr Thr Val Glu Tyr Glu Leu Lys Tyr Arg Asn Ile Gly
            65                  70                  75

AGT GAA ACA TGG AAG ACC ATC ATT ACT AAG AAT CTA CAT TAC AAA GAT       288
Ser Glu Thr Trp Lys Thr Ile Ile Thr Lys Asn Leu His Tyr Lys Asp
        80                  85                  90

GGG TTT GAT CTT AAC AAG GGC ATT GAA GCG AAG ATA CAC ACG CTT TTA       336
Gly Phe Asp Leu Asn Lys Gly Ile Glu Ala Lys Ile His Thr Leu Leu
     95                  100                 105

CCA TGG CAA TGC ACA AAT GGA TCA GAA GTT CAA AGT TCC TGG GCA GAA       384
Pro Trp Gln Cys Thr Asn Gly Ser Glu Val Gln Ser Ser Trp Ala Glu
110                 115                 120                 125

ACT ACT TAT TGG ATA TCA CCA CAA GGA ATT CCA GAA ACT AAA GTT CAG       432
Thr Thr Tyr Trp Ile Ser Pro Gln Gly Ile Pro Glu Thr Lys Val Gln
                130                 135                 140

GAT ATG GAT TGC GTA TAT TAC AAT TGG CAA TAT TTA CTC TGT TCT TGG       480
Asp Met Asp Cys Val Tyr Tyr Asn Trp Gln Tyr Leu Leu Cys Ser Trp
            145                 150                 155

AAA CCT GGC ATA GGT GTA CTT CTT GAT ACC AAT TAC AAC TTG TTT TAC       528
Lys Pro Gly Ile Gly Val Leu Leu Asp Thr Asn Tyr Asn Leu Phe Tyr
        160                 165                 170
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TGG TAT GAG GGC TTG GAT CTT GCA TTA CAG TGT GTT GAT TAC ATC AAG      576
Trp Tyr Glu Gly Leu Asp Leu Ala Leu Gln Cys Val Asp Tyr Ile Lys
    175                 180                 185

GCT GAT GGA CAA AAT ATA GGA TGC AGA TTT CCC TAT TTG GAG GCA TCA      624
Ala Asp Gly Gln Asn Ile Gly Cys Arg Phe Pro Tyr Leu Glu Ala Ser
190                 195                 200                 205

GAC TAT AAA GAT TTC TAT ATT TGT GTT AAT GGA TCA TCA GAG AAC AAG      672
Asp Tyr Lys Asp Phe Tyr Ile Cys Val Asn Gly Ser Ser Glu Asn Lys
                210                 215                 220

CCT ATC AGA TCC AGT TAT TTC ACT TTT CAG CTT CAA AAT ATA GTT AAA      720
Pro Ile Arg Ser Ser Tyr Phe Thr Phe Gln Leu Gln Asn Ile Val Lys
            225                 230                 235

CCT TTG CCG CCA GTC TAT CTT ACT TTT ACT CGG GAG AGT TCA TGT GAA      768
Pro Leu Pro Pro Val Tyr Leu Thr Phe Thr Arg Glu Ser Ser Cys Glu
        240                 245                 250

ATT AAG CTG AAA TGG GGC ATA CCT TTG GGA CCT ATT CCA GCA AGG TGT      816
Ile Lys Leu Lys Trp Gly Ile Pro Leu Gly Pro Ile Pro Ala Arg Cys
    255                 260                 265

TTT GAT TAT GAA ATT GAG ATC AGA GAA GAT GAT ACT ACC TTG GTG ACT      864
Phe Asp Tyr Glu Ile Glu Ile Arg Glu Asp Asp Thr Thr Leu Val Thr
270                 275                 280                 285

GCT ACA GTT GAA AAT GAA ACA TAC ACC TTG AAA ACA ACA AAT GAA ACC      912
Ala Thr Val Glu Asn Glu Thr Tyr Thr Leu Lys Thr Thr Asn Glu Thr
                290                 295                 300

CGA CAA TTA TGC TTT GTA GTA AGA AGC AAA GTG AAT ATT TAT TGC TCA      960
Arg Gln Leu Cys Phe Val Val Arg Ser Lys Val Asn Ile Tyr Cys Ser
            305                 310                 315

GAT GAC GGA ATT TGG AGT GAG TGG AGT GAT AAA CAA TGC TGG GAA GGT     1008
Asp Asp Gly Ile Trp Ser Glu Trp Ser Asp Lys Gln Cys Trp Glu Gly
        320                 325                 330

GAA GAC CTA TCG AAG AAA ACT TTG CTA CGT TTC TGG CTA CCA TTT GGT     1056
Glu Asp Leu Ser Lys Lys Thr Leu Leu Arg Phe Trp Leu Pro Phe Gly
    335                 340                 345
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TTC ATC TTA ATA TTA GTT ATA TTT GTA ACC GGT CTG CTT TTG CGT AAG      1104
Phe Ile Leu Ile Leu Val Ile Phe Val Thr Gly Leu Leu Leu Arg Lys
350                 355                 360                 365

CCA AAC ACC TAC CCA AAA ATG ATT CCA GAA TTT TTC TGT GAT ACA TGAAGACTTT
1159
Pro Asn Thr Tyr Pro Lys Met Ile Pro Glu Phe Phe Cys Asp Thr
                370                 375                 380

CCTCTAGA                                                            1167
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(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 380 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```
Met Ala Phe Val Cys Leu Ala Ile Gly Cys Leu Tyr Thr Phe Leu Ile
 1               5                  10                  15

Ser Thr Thr Phe Gly Cys Thr Ser Ser Ser Asp Thr Glu Ile Lys Val
            20                  25                  30

Asn Pro Pro Gln Asp Phe Glu Ile Val Asp Pro Gly Tyr Leu Gly Tyr
        35                  40                  45

Leu Tyr Leu Gln Trp Gln Pro Pro Leu Ser Leu Asp His Phe Lys Glu
    50                  55                  60

Tyr Thr Val Glu Tyr Glu Leu Lys Tyr Arg Asn Ile Gly Ser Glu Thr
65                  70                  75                  80

Trp Lys Thr Ile Ile Thr Lys Asn Leu His Tyr Lys Asp Gly Phe Asp
                85                  90                  95

Leu Asn Lys Gly Ile Glu Ala Lys Ile His Thr Leu Leu Pro Trp Gln
            100                 105                 110
```

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Cys Thr Asn Gly Ser Glu Val Gln Ser Ser Trp Ala Glu Thr Thr Tyr
        115                 120                 125

Trp Ile Ser Pro Gln Gly Ile Pro Glu Thr Lys Val Gln Asp Met Asp
    130                 135                 140

Cys Val Tyr Tyr Asn Trp Gln Tyr Leu Leu Cys Ser Trp Lys Pro Gly
145                 150                 155                 160

Ile Gly Val Leu Leu Asp Thr Asn Tyr Asn Leu Phe Tyr Trp Tyr Glu
                165                 170                 175

Gly Leu Asp Leu Ala Leu Gln Cys Val Asp Tyr Ile Lys Ala Asp Gly
            180                 185                 190

Gln Asn Ile Gly Cys Arg Phe Pro Tyr Leu Glu Ala Ser Asp Tyr Lys
        195                 200                 205

Asp Phe Tyr Ile Cys Val Asn Gly Ser Ser Glu Asn Lys Pro Ile Arg
    210                 215                 220

Ser Ser Tyr Phe Thr Phe Gln Leu Gln Asn Ile Val Lys Pro Leu Pro
225                 230                 235                 240

Pro Val Tyr Leu Thr Phe Thr Arg Glu Ser Ser Cys Glu Ile Lys Leu
                245                 250                 255

Lys Trp Gly Ile Pro Leu Gly Pro Ile Pro Ala Arg Cys Phe Asp Tyr
            260                 265                 270

Glu Ile Glu Ile Arg Glu Asp Asp Thr Thr Leu Val Thr Ala Thr Val
        275                 280                 285

Glu Asn Glu Thr Tyr Thr Leu Lys Thr Thr Asn Glu Thr Arg Gln Leu
    290                 295                 300

Cys Phe Val Val Arg Ser Lys Val Asn Ile Tyr Cys Ser Asp Asp Gly
305                 310                 315                 320

Ile Trp Ser Glu Trp Ser Asp Lys Gln Cys Trp Glu Gly Glu Asp Leu
                325                 330                 335

Ser Lys Lys Thr Leu Leu Arg Phe Trp Leu Pro Phe Gly Phe Ile Leu
            340                 345                 350
```

Ile Leu Val Ile Phe Val Thr Gly Leu Leu Leu Arg Lys Pro Asn Thr
355 360 365

Tyr Pro Lys Met Ile Pro Glu Phe Phe Cys Asp Thr
370 375 380

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Trp Ser Xaa Trp Ser
1 5

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1126 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 11..1126

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

ACTTGGAGAA ATG GCT TTC GTC TAC TTG GCT ATC AGA TGC TTA TGT ACC 49
Met Ala Phe Val Tyr Leu Ala Ile Arg Cys Leu Cys Thr
1 5 10

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TTT CTG ATA AGC ACA ACA TTC GGC TAT ACT TCA ACT TCA GAC ACC GAG      97
Phe Leu Ile Ser Thr Thr Phe Gly Tyr Thr Ser Thr Ser Asp Thr Glu
     15                  20                  25

ATA AAA GTT AAC CCA CCT CAG GAT TTT GAG ATA GTG GAT CCC GGA TAT     145
Ile Lys Val Asn Pro Pro Gln Asp Phe Glu Ile Val Asp Pro Gly Tyr
 30                  35                  40                  45

TTA GGT TAT CTC TAT TTG CAA TGG CAA CCC CCA CTG TCT CTG GAT AAT     193
Leu Gly Tyr Leu Tyr Leu Gln Trp Gln Pro Pro Leu Ser Leu Asp Asn
                 50                  55                  60

TTT AAG GAA TGC ACA GTG GAA TAT GAA CTA AAA TAC CGA AAC ATT GGT     241
Phe Lys Glu Cys Thr Val Glu Tyr Glu Leu Lys Tyr Arg Asn Ile Gly
             65                  70                  75

AGT GAA ACA TGG ACG ACC ATC ATT ACT AAG AAT CTA CAT TAC AAA GAT     289
Ser Glu Thr Trp Thr Thr Ile Ile Thr Lys Asn Leu His Tyr Lys Asp
         80                  85                  90

GGG TTT GAT CTT AAC AAG GGC ATT GAA GCG AAG ATA CAC ACA CTT TTA     337
Gly Phe Asp Leu Asn Lys Gly Ile Glu Ala Lys Ile His Thr Leu Leu
     95                  100                 105

CCA TGG CAA TGC ACA AAT GGA TCA GAA GTT CAA AGT TCC TGG GCA GAA     385
Pro Trp Gln Cys Thr Asn Gly Ser Glu Val Gln Ser Ser Trp Ala Glu
110                 115                 120                 125

GCT ACT TAT TGG ATA TCG CCA CAA GGA ATT CCA GAA ACT AAA GTT CAG     433
Ala Thr Tyr Trp Ile Ser Pro Gln Gly Ile Pro Glu Thr Lys Val Gln
                130                 135                 140

GAT ATG GAT TGT GTA TAT TAC AAT TGG CAA TAT TTA CTC TGT TCT TGG     481
Asp Met Asp Cys Val Tyr Tyr Asn Trp Gln Tyr Leu Leu Cys Ser Trp
            145                 150                 155

AAA CCT GGC ATA GGT GTA CTT CTT GAT ACC AAT TAC AAC TTG TTT TAC     529
Lys Pro Gly Ile Gly Val Leu Leu Asp Thr Asn Tyr Asn Leu Phe Tyr
        160                 165                 170

TGG TAT GAG GGC TTG GAT CGT GCA TTA CAG TGT GTT GAT TAC ATC AAG     577
Trp Tyr Glu Gly Leu Asp Arg Ala Leu Gln Cys Val Asp Tyr Ile Lys
    175                 180                 185
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GTT GAT GGA CAA AAT ATT GGA TGC AGA TTT CCC TAT TTG GAG TCA TCA        625
Val Asp Gly Gln Asn Ile Gly Cys Arg Phe Pro Tyr Leu Glu Ser Ser
190                 195                 200                 205

GAC TAT AAA GAT TTC TAC ATT TGT GTT AAT GGA TCA TCA GAA ACC AAG        673
Asp Tyr Lys Asp Phe Tyr Ile Cys Val Asn Gly Ser Ser Glu Thr Lys
                210                 215                 220

CCT ATC AGA TCC AGT TAT TTC ACT TTT CAG CTT CAA AAT ATA GTT AAA        721
Pro Ile Arg Ser Ser Tyr Phe Thr Phe Gln Leu Gln Asn Ile Val Lys
            225                 230                 235

CCT TTG CCA CCA GTC TGT CTT ACT TGT ACT CAG GAG AGT TTA TAT GAA        769
Pro Leu Pro Pro Val Cys Leu Thr Cys Thr Gln Glu Ser Leu Tyr Glu
        240                 245                 250

ATT AAG CTG AAA TGG AGC ATA CCT TTG GGA CCT ATT CCA GCA AGG TGT        817
Ile Lys Leu Lys Trp Ser Ile Pro Leu Gly Pro Ile Pro Ala Arg Cys
    255                 260                 265

TTT GTT TAT GAA ATT GAG ATC AGA GAA GAT GAT ACT ACC TTG GTG ACT        865
Phe Val Tyr Glu Ile Glu Ile Arg Glu Asp Asp Thr Thr Leu Val Thr
270                 275                 280                 285

ACC ACA GTT GAA AAT GAA ACG TAC ACC TTG AAA ATA ACA AAT GAA ACC        913
Thr Thr Val Glu Asn Glu Thr Tyr Thr Leu Lys Ile Thr Asn Glu Thr
                290                 295                 300

CGA CAG TTA TGC TTT GTA GTA AGA AGC AAA GTG AAT ATT TAT TGC TCA        961
Arg Gln Leu Cys Phe Val Val Arg Ser Lys Val Asn Ile Tyr Cys Ser
            305                 310                 315

GAT GAT GGA ATT TGG AGT GAG TGG AGT GAT AAA CAA TGT TGG GAA GTT       1009
Asp Asp Gly Ile Trp Ser Glu Trp Ser Asp Lys Gln Cys Trp Glu Val
        320                 325                 330

GAA GAA CTA TTG AAG AAA ACT TTG CTA CTT TTC TTG TTA CCA TTT GGT       1057
Glu Glu Leu Leu Lys Lys Thr Leu Leu Leu Phe Leu Leu Pro Phe Gly
    335                 340                 345

TTC ATA TTA ATA TTA GTT ATA TTT GTA ACC GGT CTG CTT TTG TGT AAG       1105
Phe Ile Leu Ile Leu Val Ile Phe Val Thr Gly Leu Leu Leu Cys Lys
350                 355                 360                 365
```

AGA GAC AGC TAC CCG AAA ATG 1126
Arg Asp Ser Tyr Pro Lys Met
370

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 372 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Met Ala Phe Val Tyr Leu Ala Ile Arg Cys Leu Cys Thr Phe Leu Ile
1 5 10 15

Ser Thr Thr Phe Gly Tyr Thr Ser Thr Ser Asp Thr Glu Ile Lys Val
20 25 30

Asn Pro Pro Gln Asp Phe Glu Ile Val Asp Pro Gly Tyr Leu Gly Tyr
35 40 45

Leu Tyr Leu Gln Trp Gln Pro Pro Leu Ser Leu Asp Asn Phe Lys Glu
50 55 60

Cys Thr Val Glu Tyr Glu Leu Lys Tyr Arg Asn Ile Gly Ser Glu Thr
65 70 75 80

Trp Thr Thr Ile Ile Thr Lys Asn Leu His Tyr Lys Asp Gly Phe Asp
85 90 95

Leu Asn Lys Gly Ile Glu Ala Lys Ile His Thr Leu Leu Pro Trp Gln
100 105 110

Cys Thr Asn Gly Ser Glu Val Gln Ser Ser Trp Ala Glu Ala Thr Tyr
115 120 125

Trp Ile Ser Pro Gln Gly Ile Pro Glu Thr Lys Val Gln Asp Met Asp
130 135 140

Cys Val Tyr Tyr Asn Trp Gln Tyr Leu Leu Cys Ser Trp Lys Pro Gly
145 150 155 160

Ile Gly Val Leu Leu Asp Thr Asn Tyr Asn Leu Phe Tyr Trp Tyr Glu
165 170 175

Gly Leu Asp Arg Ala Leu Gln Cys Val Asp Tyr Ile Lys Val Asp Gly
180 185 190

Gln Asn Ile Gly Cys Arg Phe Pro Tyr Leu Glu Ser Ser Asp Tyr Lys
195 200 205

Asp Phe Tyr Ile Cys Val Asn Gly Ser Ser Glu Thr Lys Pro Ile Arg
210 215 220

Ser Ser Tyr Phe Thr Phe Gln Leu Gln Asn Ile Val Lys Pro Leu Pro
225 230 235 240

Pro Val Cys Leu Thr Cys Thr Gln Glu Ser Leu Tyr Glu Ile Lys Leu
245 250 255

Lys Trp Ser Ile Pro Leu Gly Pro Ile Pro Ala Arg Cys Phe Val Tyr
260 265 270

Glu Ile Glu Ile Arg Glu Asp Asp Thr Thr Leu Val Thr Thr Thr Val
275 280 285

Glu Asn Glu Thr Tyr Thr Leu Lys Ile Thr Asn Glu Thr Arg Gln Leu
290 295 300

Cys Phe Val Val Arg Ser Lys Val Asn Ile Tyr Cys Ser Asp Asp Gly
305 310 315 320

Ile Trp Ser Glu Trp Ser Asp Lys Gln Cys Trp Glu Val Glu Glu Leu
325 330 335

Leu Lys Lys Thr Leu Leu Leu Phe Leu Leu Pro Phe Gly Phe Ile Leu
340 345 350

Ile Leu Val Ile Phe Val Thr Gly Leu Leu Leu Cys Lys Arg Asp Ser
355 360 365

Tyr Pro Lys Met
370

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(vii) IMMEDIATE SOURCE:
(B) CLONE: ZG9801

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

TGGTCCTTCC CATGTTTCAC TACCA 25

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(vii) IMMEDIATE SOURCE:
(B) CLONE: ZG9941

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

TTTCGGTATT TTAGTTCATA TTCCA 25

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(vii) IMMEDIATE SOURCE:
(B) CLONE: ZG9803

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

CGGAATTTGG AGTGAGTGGA GTGAT 25

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(vii) IMMEDIATE SOURCE:
(B) CLONE: ZG9937

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

TGAAGACCTA TCGAAGAAAA CTTTG 25

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(vii) IMMEDIATE SOURCE:
(B) CLONE: ZG9800

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

ATGGCTTTCG TTTGCTTGGC TATCG 25

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(vii) IMMEDIATE SOURCE:
(B) CLONE: ZG9802

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

CTCTTGATAT GGAAAGTCTT CATGTATC 28

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(vii) IMMEDIATE SOURCE:
(B) CLONE: AP1

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

CCATCCTAAT ACGACTCACT ATAGGGC 27

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(vii) IMMEDIATE SOURCE:
(B) CLONE: AP2

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

ACTCACTATA GGGCTCGAGC GGC 23

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(vii) IMMEDIATE SOURCE:
(B) CLONE: ZG9850

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

TCTGATAGGC TTGTTCTCTG 20

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(vii) IMMEDIATE SOURCE:
(B) CLONE: ZG9851

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

ATAGCCAAGC AAACGAAAGC 20

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(vii) IMMEDIATE SOURCE:
(B) CLONE: ZG9852

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

ACCTGGCATA GGTGTACTTC 20

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(vii) IMMEDIATE SOURCE:
(B) CLONE: ZG9919

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

TTGCCGCCAG TCTATCTTAC 20

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 34 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(vii) IMMEDIATE SOURCE:
(B) CLONE: ZG10317

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

GGGGGGTCTA GAGGAAAGTC TTCATGTATC ACAG 34

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(vii) IMMEDIATE SOURCE:
(B) CLONE: ZG10319

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

GGGGGGCTGG AGCTCGGAGA AATGGCTTTC GTT 33

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(vii) IMMEDIATE SOURCE:
(B) CLONE: ZG9820

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

ACCCCCACTG TCTCTGGATC ATTTT 25

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(vii) IMMEDIATE SOURCE:
(B) CLONE: ZG9806

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

CACCTTCCCA GCATTGTTTA TCACT 25

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(vii) IMMEDIATE SOURCE:
(B) CLONE: ZG10320

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

GGGGGGAGAT CTTCAGACAC CGAGATAAAA GTT 33

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(vii) IMMEDIATE SOURCE:
(B) CLONE: ZG10318

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

GGGGGGCTCG AGTTTCTTCG ATAGGTCTTC ACC 33

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(vii) IMMEDIATE SOURCE:

(B) CLONE: ZG9882

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

TTACTCTGTT CTTGGAAACC TGG 23

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(vii) IMMEDIATE SOURCE:
(B) CLONE: ZG10082

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

ACTCTGTTCT TGGAAACCTG G 21

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(vii) IMMEDIATE SOURCE:
(B) CLONE: ZG10083

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

AAATGAAACA TACACCTTGA AAAC 24

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs

(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(vii) IMMEDIATE SOURCE:
(B) CLONE: ZG10081

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

GCATTGTTTA TCACTCCACT C 21

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(vii) IMMEDIATE SOURCE:
(B) CLONE: ZG9881

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

TTCACTTTGC TTCTTACTAC AAA 23

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 39 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(vii) IMMEDIATE SOURCE:
(B) CLONE: ZG10389

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

GACTAGCAGA TCTGGGCTCT TTCTTCGATA GGTCTTCAC 39

(2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(vii) IMMEDIATE SOURCE:
(B) CLONE: ZG10314

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

TCGTGATTCT CTGGTCGGTG 20

(2) INFORMATION FOR SEQ ID NO:33:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(vii) IMMEDIATE SOURCE:
(B) CLONE: ZG10315

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

GTGATTGCTT TGGCGGTGAG 20

Claims

We claim:

1. An isolated polynucleotide encoding a ligand-binding receptor polypeptide, said polypeptide comprising a sequence of amino acids selected from the group consisting of:

(a) residues 141 to 337 of SEQ ID NO:2;

(b) allelic variants of (a); and

(c) sequences that are at least 80% identical to (a) or (b).

2. An isolated polypeptide according to claim 1 comprising residues 141 to 337 of SEQ ID NO:2 or SEQ ID NO:4.

3. An isolated polynucleotide according to claim 1 wherein said polypeptide further comprises a transmembrane domain.

4. An isolated polynucleotide according to claim 3 wherein said transmembrane domain comprises residues 340 to 363 of SEQ ID NO:2, or an allelic variant thereof.

5. An isolated polynucleotide according to claim 3 wherein said polypeptide further comprises an intracellular domain.

6. An isolated polynucleotide according to claim 5 wherein said intracellular domain comprises residues 365 to 380 of SEQ ID NO:2, or an allelic variant thereof.

7. An isolated polynucleotide according to claim 1 wherein said polypeptide comprises residues 25 to 337 of SEQ ID NO:2 or SEQ ID NO:4.

8. An isolated polynucleotide according to claim 1 wherein said polypeptide comprises residues 1 to 380 of SEQ ID NO:2 or SEQ ID NO:4.

9. An isolated polynucleotide according to claim 1 which is a DNA as shown in SEQ ID NO:1 from nucleotide 49 to nucleotide 1188 or SEQ ID NO:3 from nucleotide 10 to nucleotide 1149.

10. An isolated polynucleotide according to claim 1 wherein said polypeptide further comprises an affinity tag.

11. An isolated polynucleotide according to claim 10 wherein said affinity tag is polyhistidine, protein A, glutathione S transferase, substance P, or an immunoglobulin heavy chain constant region.

12. An isolated polynucleotide according to claim 1 wherein said polynucleotide is DNA.

13. An expression vector comprising the following operably linked elements:

a transcription promoter;

a DNA segment encoding a secretory peptide and a ligand-binding receptor polypeptide, said polypeptide comprising a sequence of amino acids selected from the group consisting of:

(a) residues 141 to 337 of SEQ ID NO:2;

(b) allelic variants of (a); and

(c) sequences that are at least 80% identical to (a) or (b); and

a transcription terminator.

14. An expression vector according to claim 13 wherein said polypeptide comprises residues 141 to 337 of SEQ ID NO:2 or SEQ ID NO:4.

15. An expression vector according to claim 13 wherein said polypeptide further comprises a transmembrane domain.

16. An expression vector according to claim 15 wherein said transmembrane domain comprises residues 340 to 363 of SEQ ID NO:2, or an allelic variant thereof.

17. An expression vector according to claim 15 wherein said polypeptide further comprises an intracellular domain.

18. An expression vector according to claim 17 wherein said intracellular domain comprises residues 364 to 380 of SEQ ID NO:2, or an allelic variant thereof.

19. An expression vector according to claim 13 wherein said polypeptide comprises residues 25 to 337 of SEQ ID NO:2 or SEQ ID NO:4.

20. An expression vector according to claim 13 wherein said polypeptide comprises residues 1 to 380 of SEQ ID NO:2 or SEQ ID NO:4.

21. An expression vector comprising the following operably linked elements:

(a) a transcription promoter;

(b) a DNA segment encoding a secretory peptide and a chimeric polypeptide, wherein said chimeric polypeptide consists essentially of a first portion and a second portion joined by a peptide bond, said first portion consisting essentially of a ligand binding domain of a receptor polypeptide selected from the group consisting of:

(i) a receptor polypeptide as shown in SEQ ID NO:2;

(ii) allelic variants of SEQ ID NO:2; and

(iii) receptor polypeptides that are at least 80% identical to (i) or (ii),
and said second portion consisting essentially of an affinity tag; and

(c) a transcription terminator.

22. An expression vector according to claim 21 wherein said affinity tag is an immunoglobulin F_c polypeptide.

23. A cultured eukaryotic cell into which has been introduced an expression vector according to claim 13, wherein said cell expresses a receptor polypeptide encoded by the DNA segment.

24. A cell according to claim 23 wherein said cell further expresses a hematopoietic receptor β_c subunit.

25. A cell according to claim 23 wherein said cell is dependent upon an exogenously supplied hematopoietic growth factor for proliferation.

26. An isolated polypeptide comprising a segment selected from the group consisting of:

(a) residues 141 to 337 of SEQ ID NO:2;

(b) allelic variants of (a); and

(c) sequences that are at least 80% identical to (a) or (b),
wherein said polypeptide is substantially free of transmembrane and intracellular domains ordinarily associated with hematopoietic receptors.

27. A polypeptide according to claim 26 further comprising an immunoglobulin F_c polypeptide.

28. A polypeptide according to claim 26 further comprising an affinity tag.

29. A polypeptide according to claim 28 wherein said affinity tag is polyhistidine, protein A, glutathione S transferase, substance P, or an immunoglobulin heavy chain constant region.

30. A polypeptide according to claim 26 that is immobilized on a solid support.

31. A chimeric polypeptide consisting essentially of a first portion and a second portion joined by a peptide bond, said first portion consisting essentially of a ligand binding domain of a receptor polypeptide selected from the group consisting of:

(a) a receptor polypeptide as shown in SEQ ID NO:2;

(b) allelic variants of SEQ ID NO:2; and

(c) receptor polypeptides that are at least 80% identical to (a) or (b),

and said second portion consisting essentially of an affinity tag.

32. A polypeptide according to claim 31 wherein said affinity tag is an immunoglobulin F_C polypeptide.

33. A method for detecting a ligand within a test sample, comprising contacting a test sample with a polypeptide comprising a segment selected from the group consisting of:

(a) residues 141 to 337 of SEQ ID NO:2;

(b) allelic variants of (a); and

(c) sequences that are at least 80% identical to (a) or (b),

and detecting binding of said polypeptide to ligand in the sample.

34. A method according to claim 33 wherein said polypeptide comprises residues 25 to 337 of SEQ ID NO:2 or an allelic variant of SEQ ID NO:2.

35. A method according to claim 33 wherein said polypeptide further comprises transmembrane and intracellular domains.

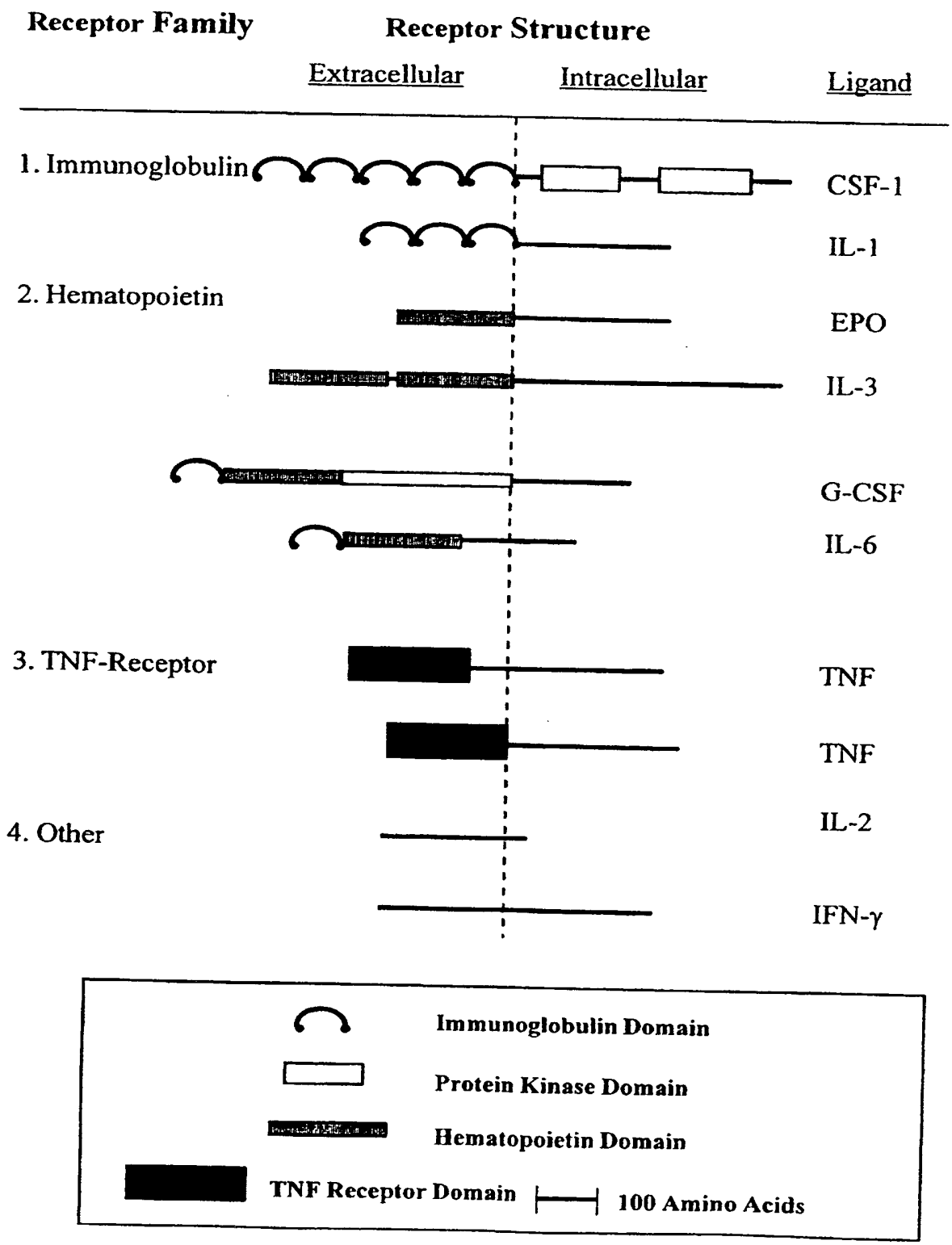
36. A method according to claim 35 wherein said polypeptide is membrane bound within a cultured cell, and said detecting step comprises measuring a biological response in said cultured cell.

37. A method according to claim 36 wherein said biological response is cell proliferation or activation of transcription of a reporter gene.

38. A method according to claim 33 wherein said polypeptide is immobilized on a solid support.

39. An antibody that specifically binds to a polypeptide of claim 26.

Figure


Receptor Family
Receptor Structure
Extracellular
Intracellular
Ligand
1. Immunoglobulin
CSF-1
IL-1
2. Hematopoietin
EPO
IL-3
G-CSF
IL-6
3. TNF-Receptor
TNF
TNF
4. Other
IL-2
IFN-γ
Immunoglobulin Domain
Protein Kinase Domain
Hematopoietin Domain
TNF Receptor Domain
100 Amino Acids

INTERNATIONAL SEARCH REPORT

Internat. Application No
PCT/US 97/04043

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07K14/715 C07K19/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 94 29458 A (AMGEN INC) 22 December 1994 see the whole document ---	1-38
Y	WO 94 19463 A (AUCKLAND UNISERVICES LTD) 1 September 1994 see abstract see page 9, line 27-31 - page 10, line 1-21 see page 12, line 1-33 ---	1-38
A	ENDOCRINOLOGY, vol. 127, no. 1, 1990, SPRINGFIELD,ILL, US, pages 251-258, XP002035642 T. TAKAO ET AL.: "Identification of interleukin-1 receptors in mouse testis" see the whole document --- -/--	1-9, 13-20, 23,26, 33-37

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

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Date of the actual completion of the international search	Date of mailing of the international search report
22 July 1997	06. 08. 97
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax (+ 31-70) 340-3016	Authorized officer Mateo Rosell, A.M.

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INTERNATIONAL SEARCH REPORT

Internat Application No
PCT/US 97/04043

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Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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PCT/US 97/04043

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

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